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*ON THE STABILITY OF NUTRITIONAL MUTANTS OF BACTERIA**

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The lactic acid bacteria are used more than any other for the quantitative determination of vitamins and amino acids. Most investigators have found that stocks of these organisms remain constant in their nutritional requirements and can be used for assay purposes year after year. Yet occasionally nutritional requirements have changed and confused assay results. For example, under the most carefully controlled experimental conditions, excellent determinations of *p*-aminobenzoic acid are not consistently obtained with *Lactobacillus arabinosus*.[1] Apparently, the requirement for *p*-aminobenzoic acid varies since it was found that cultures of this organism, which had maintained a stable requirement for more than three years, could be trained to give *p*-aminobenzoic acid-independent lines by subculturing in the presence of suboptimal concentrations of *p*-aminobenzoic acid.[2] Similarly, by subculturing in the presence of suboptimal concentrations of tryptophane, strains of *L. arabinosus* have been developed which no longer require tryptophane in the medium for growth.[3]

It was a recognition of these biological properties of bacteria which led Lloyd[4] to qualify his discussion of the use of microörganisms for the determination of amino acids. He wrote: "Microörganisms do not always behave, generation after generation, like a standard reagent, nor need their behavior be independent of the general composition of the medium." Lloyd is correct; since bacteria are organisms they exhibit the property of variation. However, we need to know what the factors are that give rise to and establish variations in the nutritional requirements of bacteria.

Mutation.—Until the method of variance analysis was applied to the study of mutation in bacteria[5] it was very difficult to discover the cause of an inherited change. For instance, the loss of a nutritional requirement through subculturing in suboptimal medium may be thought to be due to

the induction of the change in the bacteria by the chemical stimulus of the new nutrients in the absence of the normal nutrients.[6] Or it may be due to selection by the suboptimal medium of "spontaneously" occurring mutants which are independent of a supply of the substance in which the medium is deficient, and which occur regardless of the presence or absence of the substance. The former hypothesis has been favored by many students of the adaptation of nutritional requirements.[7] However, their reasons for this acceptance are usually inadequate and involve a failure to recognize that, although the mutation rate per generation of bacteria is of the same order of magnitude as that of other organisms, mutations in a growing culture are frequent because of the rapid rate of multiplication. Ivanovics and Eöllös[8] have asserted that pantothenic acid-deficient strains of a Ruhrbazillus are variants not engendered by genetic defects because they can be made independent. But on the contrary, it is just this development of independence which can be examined statistically to discriminate between the two hypotheses on the origin of changes in nutritional habit.

Bacterial reproduction is clonal. Therefore a mutation occurring early in the growth of a culture will be represented at the end of growth by more organisms of the mutant type than will represent a later occurring mutation. Mutations are rare events, often occurring with a frequency of *ca.* 10^{-8}. Mutations occurring late in the growth of a culture, when there are more bacteria, will be more numerous than those occurring early. Hence, on a random mutation hypothesis we would expect to find a tremendous variation in the number of mutant bacteria at final growth among a series of different cultures. More cultures should contain small numbers of mutants (late mutations) than large numbers (early mutations). On the induced mutation hypothesis we would expect to find little variation from culture to culture since each organism present in the final growth has a given chance of responding to the testing conditions by mutation. The variability on this hypothesis should be due to sampling error. Comparisons of the variability in the number of mutant bacteria among several independent cultures with the variability among several samples from the same culture have been made and the former has always greatly exceeded the latter. In this way it has been shown that the development of bacterial resistance to bacteriophage, to penicillin, to sulfa drugs and to ultra-violet and x-radiation is spontaneous in the sense that it is not induced by the injurious agent.[9] Likewise, the mutation of *Clostridium septicum* to a condition where it is independent of added uracil for growth, can apparently occur throughout growth in the presence of uracil.[10] However, since the latter demonstration was complicated by several technical difficulties, an examination was made of the change to histidine independence by a mutant of *Escherichia coli*.

This mutant (No. 148-334) was secured after x-radiation at the Stamford Laboratories of the American Cyanimide Co.[11] I am indebted to Drs. J. O.

Lampen and R. R. Roepke who placed it at my disposal. It requires for growth salts, glucose and the amino acid, histidine. When washed cultures are plated without dilution into a chemically defined medium and agar devoid of histidine a variable and small number of colonies is formed. These upon isolation give rise to histidine-independent ($h+$) strains whose growth rate and final yield of bacteria in the absence of histidine are the same as that of the original histidine-dependent mutant ($h-$) in its presence.[12] The $h+$ strains are stable and histidine independence is inherited in the presence or absence of histidine. In this strain of *E. coli*, where gene recombination experiments cannot be performed,[13] it is impossible to tell whether the histidine-independence that develops is due to a back mutation of the factor that makes for the histidineless character of the $h-$ strain, to a supressor mutation or to both. At any rate, the $h+$ mutants are physiologically indistinguishable from the original wild type parent strain.

Four separate experiments were performed to examine the variation in numbers of $h+$ bacteria from culture to culture. Strain 148-334 was inoculated into a tryptose-yeast extract medium and incubated at 37°C. for 12 hours. A 10^{-8} dilution of the resulting culture was prepared in chemically defined medium supplemented with 100 γ *L*-histidine monohydrochloride-$.H_2O$ per ml. This dilution resulted in a concentration of about 35 bacteria per ml. Ten 1-ml. aliquots and one 7-ml. aliquot were placed in 12-ml. Pyrex centrifuge tubes. After growth at 37° for 24 hours the cultures were centrifuged, the supernatant decanted and the bacteria were resuspended in the original volume of chemically defined minimal medium. This procedure was carried out again and a 0.5-ml. aliquot of the final suspension was taken from each of the 10 1-ml. cultures and plated without dilution into minimal medium solidified with 2 per cent washed agar. Ten 0.5-ml. samples from the 7-ml. culture were similarly plated. To determnine the total number of bacteria in each culture 1 ml. of a 10^{-7} dilution of the suspension remaining in the centrifuge tubes was plated into minimal agar supplemented with 100 γ of histidine per ml. The plates were incubated at 37° for three days and counted. The results are shown in table 1.

The variance in the number of mutant $h+$ bacteria among different samples from the same culture is always about equal to the mean number present and hence is probably due to random sampling error. In every case it is probable that the mutants were obtained from a homogeneous population. On the other hand, the variance in the number of $h+$ organisms among single samples from different cultures is many times the mean number and there is an insignificant probability that the mutants were obtained from a homogeneous population of cultures.[14] Apparently mutations occur at different times during the growth of the culture in the presence of histidine. They are not induced by the absence of histidine on

TABLE I

THE NUMBER OF HISTIDINE-INDEPENDENT BACTERIA IN SAMPLES FROM DIFFERENT CULTURES, AND IN SAMPLES FROM SINGLE CULTURES

	SAMPLES FROM DIFFERENT CULTURES — EXPERIMENT					SAMPLES FROM SINGLE CULTURES — EXPERIMENT			
	1	2	3	4		1	2	3	4
MEAN NO. OF BACTERIA PER SAMPLE ± STANDARD ERROR ($\times 10^{-7}$)	38 ± 2	23 ± 1	25 ± 5	43 ± 2		27 ± 1	32 ± 1	30 ± 3	24 ± 3
CULTURE NO.					SAMPLE NO.				
1	61	33	35	22	1	37	60	48	26
2	52	30	32	36	2	43	39	38	30
3	65	541	30	38	3	58	59	48	25
4	67	29	94	35	4	49	43	48	29
5	57	13	43	69	5	47	51	50	32
6	80	64	33	21	6	48	60	51	21
7	104	30	43	30	7	40	37	48	32
8	68	...	43	460	8	51	44	48	23
9	53	...	62	30	9	40	..	51	21
10	...	...	103	25	10	56	..	54	27
Average	67	106	52	77	..	47	49	48	27
Variance	263	31,778	694	18,334	..	51	93	18	17
χ^2	31.4	2,099	120	2,143	..	9.6	13.2	3.3	5.8
P	<0.001	<0.001	<0.001	<0.001	..	0.38	0.15	0.95	0.75

the testing plates. In this respect they are spontaneous and due to unknown and possibly randomly distributed events.[15]

Selection.—The mere occurrence of a mutation affecting nutritional habit does not guarantee its establishment in a population. Although the mutant may have a selective advantage under certain conditions it may, under other conditions, lose out in competition with its parental type.[16] Although the $h+$ and $h-$ bacteria have identical growth rates when grown separately, experiments were performed to examine the possibility of competition when the two types are together in the same culture. Strains $h-$ and $h+$ were separately grown in tryptose-yeast-extract medium at 37°C. for 17 hours. Then 2 ml. of the former and 1 ml. of the latter were mixed together. A tube containing 10 ml. of the same medium was inoculated with one loop of the mixture and incubated at 37°C. for 24 hours. Immediately after it was used for inoculation, the mixture of the two types of bacteria was diluted to 10^{-8} in minimal medium and 5 1-ml. samples were plated into minimal medium solidified with 2 per cent washed agar. When the plates had hardened a thin layer of the same agar medium was poured over the surface. The 24-hour growth of the mixture in tryptose-yeast extract medium was similarly diluted and plated. Three days after the plating $h+$ colonies appeared on both sets of plates which had been stored at 37°C. These colonies were counted and their position marked on the Petri dish with glass-marking ink. Then a layer of agar supplemented with 500 γ *L*-histidine monohydrochloride.H_2O per ml. was spread over the surface and the plates were incubated for another 3 days at 37° C. During this time the histidine from the surface layer had diffused throughout the rest of the plate and permitted the growth of the $h-$ bacteria. These appeared as new colonies whose number did not increase after the 3-day period. Comparison of the number of these new colonies with the old $h+$ colonies yielded the ratio of the two types of organisms in the culture.[17] Independent determinations of the numbers of organisms in the original cultures of $h-$ and $h+$ indicated that the mixture should contain about 48 per cent $h-$ bacteria. Direct determinations on the mixture showed that 58 per cent were present. After the mixture had grown to completion for 24 hours 44 per cent $h-$ bacteria were present. No appreciable selection of either type had taken place even though growth had occurred through about 11 generations. A second experiment was performed in which the mixture was plated before and after growth directly into minimal medium with 2 per cent washed agar and a limiting concentration of histidine (3 γ per ml.). The $h+$ organisms grew to normal-sized colonies which were easily distinguished from the very small colonies formed by the limited growth of the $h-$ bacteria. Comparisons of the numbers of large and small colonies showed that the proportion of $h-$ organisms in the mixture before growth was 82 per cent, and after growth for 24 hours, 81 per cent. Once again

there was no evidence of selection for or against the growth of either type of organism in the presence of optimal amounts of histidine.

In the absence of histidine, however, there is a strong selection in favor of $h+$ organisms. When an $h-$ culture is washed and 0.05 ml. is inoculated into medium containing optimal amounts of histidine (5–100 γ per ml.) growth is complete at 37°C. in less than 10 hours. When the same inoculum is introduced into minimal medium devoid of histidine there is no growth at 10 hours. But after about 18 hours growth does occur. This growth consists (as determined by plating) almost entirely of $h+$ bacteria which arose from the few $h+$ organisms which were present in the $h-$ culture as spontaneous mutants. In the absence of histidine they have been selected for and overgrow the culture. This is clearly shown in an experiment in which 0.05 ml. of mixtures of different proportions of washed $h-$ and $h+$ bacteria were inoculated into 10 ml. of medium with and without histidine. The composition of the inoculum and the resulting growth was determined by the first method described above. As can be seen in table 2

TABLE 2

THE EFFECT OF COMPOSITION OF THE INOCULUM ON THE PROPORTION OF HISTIDINELESS BACTERIA IN CULTURES THAT HAVE CEASED TO GROW

PER CENT $h-$ BACTERIA IN INOCULUM	PER CENT $h-$ BACTERIA AFTER GROWTH FOR 24 HOURS ON (γ HISTIDINE PER ML.)		
	25.6	0.4	0
99.1	98.9	23.0	2.1
87.8	78.4	5.3	2.7
54.5	43.9	3.7	1.5
49.6	31.9	2.1	0.5
6.9	9.1	1.6	<0.1

the growth that occurs in the presence of an optimal amount of histidine (25 γ per ml.) does not substantially change the proportion of $h+$ to $h-$ bacteria. (This proportion does not shift even after the culture has been in the stationary phase for more than 60 hours.) However, when growth of the mixture occurs in a histidine concentration (0.4 γ per ml.) that is limiting for the $h-$ bacteria the $h+$ organisms are selected for and the per cent $h-$ bacteria in the growth culture is less than that of the inoculum. The selective advantage of the $h+$ organisms is even greater when the mixtures are inoculated into medium devoid of histidine. The small fraction of $h-$ bacteria which are present after final growth are probably supported by traces of histidine in the minimal medium and by histidine secured from the growing $h+$ bacteria. It may be concluded that the $h-$ organisms lose out in competition with $h+$ forms in the absence of histidine, while in the presence of an optimal amount neither type seems to have an advantage.

Population Equilibrium.—Ideally the artificial mixture experiment should consist of the introduction of a single $h+$ bacterium into a very large

number of $h-$ organisms (*ca.* 10^6–10^7) in order to mimic the event of mutation. This, however, is not feasible because the variable number of $h+$ bacteria which are added by "random" mutation cannot be distinguished from the progeny of the introduced $h+$ bacterium without the use of special markers. Additional evidence, however, that there is no positive selection for $h+$ organisms in the presence of optimal histidine comes from the determination of the frequency of $h+$ bacteria in cultures grown from serially transferred nutrient agar slants. These determinations, shown in table 3, were made over a period of 3 years for other purposes and are not

TABLE 3

THE EFFECT OF SERIAL TRANSFER AND AGING OF $h-$ CULTURES GROWN ON AGAR SLANTS ON THE PROPORTION OF $h+$ BACTERIA IN LIQUID CULTURES ESTABLISHED FROM THEM

NO. OF SERIAL TRANSFER	AGE OF SLANT (MONTHS)	NO. OF $h+$ BACTERIA PER 10^8 ORGANISMS
0	1.0	0.17
0	2.7	0.16
0	3.0	0.16
0	4.0	0.11
8	1.4	0.10
8	2.0	0.45
8	2.1	0.88
8	8.4	0.28
9	1.5	0.42
10	0.1	0.15
11	0.1	0.17
13	1.8	0.21
13	2.4	0.59
13	3.4	0.42
13	6.4	0.35
15	4.7	0.24
15	6.4	0.28

direct determinations of the proportion of $h+$ bacteria in the growth on the agar slants. Inocula from these slants, containing about 10^7 bacteria, were in each case introduced into medium containing optimal histidine (25 γ per ml.) and after growth was complete the proportion of $h+$ bacteria was determined by plating. It can be seen that there is no trend in the values so obtained; the variance is like that due to mutation during growth (see table 1). This suggests that the different stock slants did not contain significantly different proportions of $h+$ organisms. The serial transfers of one stock culture to another were made about every 2 months, and since each slant was established from about 10^7 bacteria and contained about 8×10^9 bacteria some 10 generations were involved in its growth. There is no evidence for a progressive selection in favor of the $h+$ bacteria during the 150

generations which ensued in the course of the 15 transfers. Indeed, direct platings of a 4.2-month-old 16th transfer and of a 2-day-old 17th transfer showed that they contained 0.18 and 0.15 $h+$ bacteria per 10^6 bacteria. These ratios are essentially the same as the values in table 3 and indicate that the proportion does not appreciably change on a slant and that it does not change from slant to slant after serial transfers.

Apparently an equilibrium proportion had been reached in the serially transferred stock slants. Such an equilibrium could be achieved by the balance of forward and backward mutations, selection pressure or both. An analysis of the assumption of this equilibrium will be presented in another communication. For the present it may securely be stated that in the presence of optimal concentrations of histidine there is no selection in favor of $h+$ bacteria.

Discussion.—The spontaneous nature of the mutation from $h-$ to $h+$ is further evidence that the nutritional characteristics of bacteria are controlled by genes which have mutability properties similar to those of sexual organisms, like the mold Neurospora.[16] Just as in organisms where mutants induced in the laboratory by x-rays or ultra-violet radiation are similar to those occurring spontaneously and found in nature, so naturally occurring strains of bacteria, like *L. arabinosus* probably have their nutritional requirements controlled by mutable genes. The Lindegrens[18] have found that naturally occurring yeasts have nutritional requirements that are due to single mutable genes. Such growth-factor deficiencies are probably due to spontaneous mutations and, similarly, the development of the ability to dispense with previously required growth factors must, in many cases, be due to spontaneous mutation.

Some nutritional requirements seem more stable than others. This may be due to a favorable selection phenomenon, to an inherent stability of the gene concerned, to the presence or absence of other mutator genes, or to the fact that the synthesis of the substance required is blocked at different steps by mutations in several genes. In the last case the chance that back mutations would occur simultaneously in two or more genes becomes extremely small. Advantage of this fact has been taken in developing a strain of Neurospora whose leucine synthesis is blocked at two steps by two different mutant genes. Although when each gene is present by itself in a separate strain adaptation is not infrequent, the double mutant has not been known to adapt in our laboratory. In bacteria, where adaptation to drug resistance has been shown to depend upon gene mutation in some instances,[9] the simultaneous development of resistance to several different drugs is known to occur so rarely as to be negligible.[19]

Although the rate of mutation is critical in determining the stability of a nutritional requirement, selection may exaggerate its influence in an explosive way. In the presence of a limiting amount of growth factor a

culture may be rapidly overgrown by a spontaneously occurring growth factor-independent mutant. For example, by continuously subculturing in the presence of suboptimal concentrations of tryptophane we, like Wright and Skeggs,[3] have obtained tryptophane-independent mutants of *L. arabinosus*. Yet these mutants never overgrow cultures maintained in the presence of optimal concentrations of tryptophane. This is a common situation. In the presence of an optimal concentration of growth factor there may be no selection for or against a deficient type. However, selection is not always unimportant in the establishment of deficient mutant types in a population. For example, the adaptation of a mutant of Neurospora, which is used for the determination of leucine, has been shown to be due to back mutation of the leucineless gene to a condition where it enables the synthesis of leucine. Although the leucine-independent mutant has a selective advantage in the absence of leucine, in the presence of leucine the leucine-dependent mutant has a selective advantage.[16] This competition is not due to a simple difference in growth rates although this may sometimes be the case[11, 20]. Either of these mechanisms may have been responsible for the phenomenon found in *Bacterium typhosum*.[21] A strain of this organism was originally able to utilize ammonia as the sole source of nitrogen. However, after serial transfer on nutrient agar (which contains tryptophane) this ability was lost. An examination of the original ammonia-utilizing culture showed that some organisms were already present which required nitrogen in organic form. It was subsequently shown that strains of *B. typhosum* unable to utilize ammonia required tryptophane.[22] There had probably been a selection of tryptophane-dependent bacteria on nutrient agar in the course of the "training."

We may conclude, then, that there are two main factors which must be controlled to keep a culture stable—mutation and selection. The usual methods employed in maintaining stock cultures of bacteria are in most instances manifestly satisfactory. The retention of cultures in the stationary phase of growth in the cold is certainly not conducive to increased mutation. The more growth that is allowed the greater the chance that a mutation will occur. In view of this it may sometimes be wise to grow no more bacteria in stock tubes or in inocula cultures than are needed. For the same reason lyophilized stock cultures are advantageous when the process itself is known to have no influence on mutation or selection. It may also sometimes be helpful to avoid taking samples for inocula directly from the surface of a slant for mutants, when they appear, will be concentrated in the region of their formation. A small sample of a suspension of the contents of a slant surface will have a lesser chance of containing a large number of mutants. In addition, a strain of bacteria with a nutritional requirement ought to be periodically purified by plating and colony isolation.

More important, perhaps, than methods for decreasing mutation are

methods for preventing the selection of mutants once they occur. This can best be achieved by being certain that an optimum level of the required growth factor is used in making medium for stock cultures. The use of "complete" media containing mixtures of natural products may not always be satisfactory for this purpose even though the medium is known to contain an optimum amount of the growth factor. For example, arginine is known to specifically inhibit a lysineless mutant of Neurospora.[23] Hence it may be advisable in some cases to use for stock tubes chemically defined medium supplemented with known amounts of growth factor. In most cases the growth factor-dependent organisms will either not be selected against or will have a selective advantage under such conditions. This is probably the mechanism which operates in nature to maintain nutritionally deficient strains.

Summary.—Mutation and selection have been shown to account for the instability of a histidineless strain of *E. coli*. The loss of the requirement for histidine is not induced by the absence of histidine but occurs during the growth of a culture in its presence. Histidine-independent mutants are selected for in the absence (or in the presence of limiting concentrations) of histidine but not in the presence of optimal concentrations. Similar situations are apparently widespread and the maintenance of a stable nutritionally deficient strain of bacteria must take these factors into consideration.

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[1] Williams, R. J., Eppright, M. A., Cunningham, E., and Mills, C. A., *Arch. Biochem.*, **5**, 299–306 (1944).

[2] Pennington, D., *Science*, **103**, 397 (1946).

[3] Wright, L. D., and Skeggs, H. R., *J. Biol. Chem.*, **159**, 611–616 (1945).

[4] Lloyd, D. J., *Nature*, **157**, 426–427 (1946).

[5] Luria, S. E., and Delbrück, M., *Genetics*, **28**, 491–511 (1943).

[6] Knight, B. C. J. G., Bacterial Nutrition, British Medical Research Council, Special Report Series, No. 210, His Majesty's Stationery Office, London, 1936.

[7] Postgate, J. R., and Hinshelwood, C. N., *Trans. Farad. Soc.*, **42**, 45–56 (1946); Hinshelwood, C. N., *The Chemical Kinetics of the Bacterial Cell*, Clarendon Press, Oxford, 1946.

[8] Ivanovics, G., and Eöllös, Z., *Schweiz. Z. Path. Bakt.*, **7**, 73–86(1944).

[9] Demerec, M., these PROCEEDINGS, **31**, 16–24 (1945); Demerec, M., *Ann. Miss. Bot. Gard.*, **32**, 131–138 (1945); Luria, S. E., *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 130–138 (1946); Oakberg, E. F., and Luria, S. E., *Genetics*, **32**, 249–261 (1947); Witkin, E. M., these PROCEEDINGS, **32**, 59–68 (1946); Witkin, E. M., *Genetics*, **32**, 221–248 (1947).

[10] Ryan, F. J., Schneider, L. K., and Ballentine, R., these PROCEEDINGS, **32**, 261–271 (1946).

[11] Roepke, R. R., Libby, R. L., and Small, M. H., *J. Bact.*, **48**, 401–412 (1944).

[12] Ryan, F. J., and Schneider, L. K., *Genetics*, in press; Ryan, F. J., and Schneider, L. K., *J. Bact.*, in press.

[13] Lederberg, J., *Genetics*, **32**, 505–525 (1947).

[14] It should be noted that these data were secured by the plating of undiluted *h*− cultures. When such cultures are diluted and plated the *h*− bacteria form microcolonies which are not visible to the naked eye. Mutations to the *h*+ condition occur in these microcolonies and in some cases this results in the formation of visible *h*+ colonies. These plate mutations obscure the characterization of the number of *h*+ organisms originally present in the *h*− culture. They can be eliminated by omitting asparagine from the minimal medium. Asparagine was present in the medium used for the experiments reported in this paper. Although some plate mutations occur under the crowded conditions formed by plating undiluted cultures, their number is insignificantly small. Even after 5 days less than 5 per cent of all *h*+ colonies are formed by plate mutation, a source of error well within that attributable to random sampling (Ryan, F. J., and Schneider, L. K., *Genetics*, in press). It is interesting to note that a variance analysis of the number of *h*+ colonies on plates of 10^{-6} dilutions of different *h*− cultures indicates that they form a homogeneous population ($P = 0.54$; P for the variance in the number of *h*+ colonies on plates of different samples of the same culture = 0.51). This is independent evidence that 10^{-6} dilutions of *h*− cultures contain no *h*+ organisms but that *h*+ colonies formed on plates of such dilutions are derived from events occurring on the plates.

[15] The spontaneous mutations may conceivably be to a condition of susceptibility to respond to the testing conditions. This notion is operationally the same as mutation to the ability the testing conditions demonstrate.

[16] Ryan, F. J., *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 215–227 (1946).

[17] Lederberg, J., and Tatum, E. L., *J. Biol. Chem.*, **165**, 381–382 (1946); see also reference 12. Under the conditions of this experiment microcolony mutation adds an insignificantly small number of *h*+ colonies.

[18] Lindegren, C. C., and Lindegren, G., *Science*, **102**, 33–34 (1945).

[19] Carpenter, C. M., Bahn, J. M., Ackerman, H., and Stokinger, H. E., *Proc. Soc. Exp. Biol. Med.*, **60**, 168–171 (1945); Sullivan, M., Stahly, G. L., Burkeland, J. M., and Myers, W. G., *Science*, **104**, 397–398 (1946).

[20] Monod, J., *Ann. Inst. Past.*, **72**, 879–890 (1946).

[21] van Loghem, J. J., *Zbl. Bakt. I. Abt. Orig.*, **57**, 385–387 (1911).

[22] Fildes, P., Gladstone, G. P., and Knight, B. C. J. G., *Brit. J. Exp. Path.*, **14**, 189–196 (1933).

[23] Doermann, A. H., *Arch. Biochem.*, **5**, 373–384 (1944).

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*A METHOD FOR SELECTION OF BIOCHEMICAL MUTANTS OF NEUROSPORA**

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Numerous investigations in recent years have firmly established the value of mutants of the mold Neurospora as tools for research in biochemistry and genetics.[1, 5, 6] Although various means have been used for inducing mutations,[4, 5, 6] the technique of isolation as described by Beadle and

Tatum[4] has been used by most investigators. This method involves isolation of single ascospores, one from each perithecium, derived from a cross of which one parent was treated. By this method at least 97 per cent of the cultures obtained from the single spore isolations are unchanged wild type strains. In order to obtain specific mutants or a diversity of mutants, it is therefore necessary to isolate a very large number of single ascospores.

The present investigations were undertaken for the purpose of developing a method for obtaining desired mutants with less effort than that required by the previous method of single ascospore isolations.

It was obviously desirable to develop a technique for eliminating a large proportion of wild type strains from those isolated and tested. Such a technique has been developed by Fries, for Ophiostoma.[7] An attempt was made to apply a modified form of the Fries technique to Neurospora by removing non-mutated strains by continuous filtration of a suspension of irradiated microconidia that were allowed to germinate and grow on a medium that would support growth of wild type strains but not mutant strains. This procedure was not a success due to a rapid loss in viability of the microconidia used, and because of clogging of the filter by growing mycelia. The few mutants that were obtained by this method occurred as heterocarotic mixtures with wild types.

A successful method has been developed, based on a visual selection of mutants among ascospores germinating on a minimal medium that will support wild type growth but not the growth of biochemical mutants. By this procedure about 80 to 95 per cent of the wild type spores can be discarded without testing.

A major consideration in developing a method of selecting specific mutants is concerned with the effect of a single mutation on the total metabolic pattern of the new strain. There is now ample evidence that the growth of some mutants is inhibited by metabolites which do not affect wild type strains. An outstanding example that has been described in detail is that of the large group of lysineless mutants of Neurospora.[8] These mutants are all inhibited by arginine. It was therefore thought desirable to make the isolation on minimal medium supplemented only with the metabolite that the desired mutant requires for growth. The new method of obtaining mutants by isolating germinating ascospores has been subjected to three types of experiments in order to provide evidence as to its practicability.

1. Germinating mutant ascospores were picked from crosses between known mutants and wild type.

2. A sample of miscellaneous mutant strains (i.e., requiring an amino acid, vitamin, purine or pyrimidine) was picked from a cross of irradiated wild type and untreated wild type.

3. Using appropriate media, specific types of mutants were picked from a cross of irradiated wild type and untreated wild type.

Experimental.—Media: In the experimental work to be described, all crosses were made on the synthetic medium described by Westergaard and Mitchell.[9] This medium supports the formation of a much larger number of perithecia than does corn meal agar.

The "minimal" medium utilized has been described by Beadle and Tatum.[4] The "complete" medium, however, has been modified using Neurospora extract rather than yeast extract and malt extract. This was prepared from Neurospora strain, Abbott 4A, grown with aeration for 3.5 days in 15 liters of minimal medium. The mycelium was collected, chopped and suspended in 4 parts of water. After 20 hours' autolysis under toluene at 37°C., the suspension was autoclaved and filtered. Hydrolyzed casein was added to the filtrate to a concentration of 0.5 mg. %. This preparation was used either as a liquid or solidified with 1.5% agar.

Selection Method: Petri plates (100 mm.) containing 25 ml. of agar medium[9] were inoculated by spreading a suspension of conidia of one mating type of Neurospora over the surface. Following formation of protoperithecia (4 to 6 days) crosses were made by adding a suspension of conidia of the opposite mating type. In cases where irradiation was carried out, the conidia of the second mating type were obtained from a six-day-old culture of wild type grown on minimal medium. These spores were suspended in sterile distilled water and exposed for 4 minutes at a distance of 3.5 in., to radiation from an "Americanaire" ultra-violet lamp (American Sterilizer Co.). More than 95 per cent of the conidia were killed by the treatment. The rayed conidia from one test tube culture were spread over two plates of protoperithecia, producing on incubation, from 1000 to 2000 perithecia. After about 11 days spores were emitted spontaneously and samples were collected on plates containing 25 ml. of medium solidified with 4 per cent agar. Collections were made by inverting the plate containing perithecia over the second plate and thereby allowing the spores to fall on the agar surface. Examinations under the microscope were made at intervals and the collection stopped when the spores were, roughly, 1 mm. apart. The time required to obtain such a distribution varied from 5 to 30 minutes. The plate was then covered and placed at once in an oven at 60°C. for 30 minutes in order to activate the ascospores. This treatment serves also to kill conidia which are usually present. After 12 hours' incubation at 25°C., the wild type ascospores had produced mycelia covering an area about 1–2 mm. in diameter while the mutants were of the order of one-tenth to one-twentieth the size of wild type. Since each perithecium sheds spores into a localized region, the presence of clusters of small forms facilitated detection of mutants. Only one mutant ascospore was taken from a cluster to avoid excessive duplication. The presumed mutant ascospores were picked up on small blocks of agar by use of fine pointed platinum-iridium spatula. They were then placed in a small test tube con-

taining the desired medium. The cultures obtained in this manner were then tested and the mutants identified in much the same manner as that described by Beadle and Tatum.[4]

Selection of Known Mutants: In order to test the method of visual selection of mutants from germinating ascospores, individual crosses were made between wild type and four different known mutants. Ascospores from the perithecia of these crosses were collected on plates of minimal agar and an attempt was made to pick the mutant types. Since in these plates the ratio of mutants to wild type strains was approximately one to one, the task was much simpler than it would be using rayed material where the incidence of mutants is of the order of 2 per cent. Data from this experiment are given in table 1.

TABLE 1

THE VISUAL SELECTION OF MUTANTS FROM CROSSES BETWEEN KNOWN MUTANT AND WILD TYPE STRAINS

MUTANT REQUIREMENT	NO. PICKED	CULTURES OBTAINED	VIABLE SPORES (%)	NO. OF MUTANTS	MUTANTS (%)
Patothenic Acid	45	16	36	16	100
Uridine	55	45	82	45	100
Adenine	55	39	71	38	97
Lysine	45	20	44	18	90

The data of table 1 demonstrate that the method of visual selection of mutants is basically sound.

General Selection of Mutants: A cross was prepared in four plates, using protoperithecia of *Neurospora crassa* 6a and irradiated conidia of 3A. These strains were newly reisolated from a cross of Em5256A and Em 5297a. Sphores were shed 11 days after the cross was made and collections on plates of minimal agar were begun at this time. These collections were made each day for 7 days with one final sample taken on the 14th day. The shedding of spores was nearly complete at this time. Approximately 20 germinated spores were picked from each plate at each collection and transferred to tubes of casein, Neurospora extract, agar medium. All of the resulting cultures were tested on minimal medium and those that grew in 3 days were discarded. The remaining cultures were tested on the classification mixtures previously described:[4] (1) vitamins; (2) amino acids; (3) yeast extract and (4) minimal. An additional mixture was also introduced to facilitate classification of the more common types of mutants previously obtained. This mixture contained methionine, 2.5 mg. %; adenine, 5 mg %; cytidine, 3 mg. %; lysine, 10 mg. %; tryptophane, 5 mg. %; and succinic acid, 10 mg. %.

Four kinds of mutants were obtained; those which did not grow on minimal medium but grew when supplied yeast extract or specific known compounds; those which grew very slowly on minimal and complete

media; those which covered the surface of the agar in the tubes but grew no further regardless of the supplement given; and those which were obviously morphological (mostly colonial) mutants. Mutants of the last two categories are not reported here. In addition, mutants collected from the same plate on the same day and which required the same known compound were considered to be duplicates. Only one representative was tabulated. Actually very few cases of such duplications were found.

The results of this experiment are summarized in table 2. Two mutant types are recorded: slow growing mutants and biochemical mutants. The requirements of the majority of the biochemical mutants are also given. In many cases these mutants grew only on the yeast extract classification mixture and their specific requirements were not determined. These are listed as unknown mutants.

TABLE 2

SELECTION OF SLOW GROWING AND BIOCHEMICAL MUTANTS

PLATE NO.	NO. PICKED	CULTURES OBTAINED	NO. SLOW MUTANTS	NO. BIOCHEMICAL MUTANTS	TYPES OF BIOCHEMICAL MUTANTS
1	112	71	3	6	Threonine, arginine methionone, cytidine, unknown (2)
2	163	61	9	8	Proline, cytidine, *p*-aminobenzoic acid (2), unknown (4)
3	114	62	3	8	Cytidine, methionine, arginine, adenine (2), unknown (3)
4	123	55	12	12	Lysine, methionine, adenine + methionine, tryptophane (2), unknown (2)
Total	512	249	27	34	

It may be observed from the data in table 2 that a wide variety of mutants was obtained from the four plates. Biochemical mutants which did not grow on minimal medium but did grow in the presence of an appropriate supplement constituted 13 per cent of the cultures that were obtained.

Selection of Specific Mutants: Several experiments were designed to select specific mutants from 20 plates prepared as described in the previous section. The first of these was concerned with selection of mutants requiring histidine. No histidineless mutants have been obtained previously in Neurospora. Ascospores were collected on minimal medium and, after germination, probable mutants were transferred to minimal medium containing 5 mg. % histidine monohydrochloride. Resulting cultures, 47 from 115 spores picked, were tested on minimal. One failed to grow and it was subsequently established to be a histidineless mutant. This mutant fails to grow in the presence of hydrolyzed casein, yeast extract or Neurospora ex-

tract in the concentrations usually used in complete medium. It seems probable that mutants of this type occurred but were eliminated in previous tests because of their failure to grow on the complete medium used.

In a second experiment an attempt was made to select for three types of mutants simultaneously. These were: a frequently occurring type requiring adenine; a type that has occurred only infrequently, requiring pantothenic acid; and a specific mutant, not previously found, that cannot condense indole and serine to form tryptophane. Mutants of the third type might be expected to lack the enzyme which links serine to indole. The presence of this enzyme in wild type Neurospora has been reported by Umbreit, *et al.*[10] In order to minimize the selection of other mutants the ascospores were collected on plates of minimal agar supplemented with the following metabolites: charcoal treated, acid hydrolyzed casein, 100 mg. %; riboflavin, 0.5 mg. %; thiamin, 0.25 mg. %; pyidoxine, 0.25 mg. %; cytidine, 3.75 mg. %; indole 5.0 mg. %. These substances were added to minimize the picking of undesired mutant strains. Presumably mutants having these metabolites as growth requirements would be indistinguishable from wild type strains on the plates.

Of 381 ascospores picked from these plates, 265 grew when placed on a medium of minimal agar supplemented with 5 mg. % adenine, 5 mg. % tryptophane and 0.5 mg. % pantothenic acid. On testing the cultures that grew in this medium, it was found that there were no adenine or pantothenic acid mutants but that one tryptophane mutant had been selected. This mutant required tryptophane for growth but did not grow when supplied indole. It was consequently one of the mutants for which the selection was made. No adenine mutants were found in this experiment in spite of the fact that they have occurred at fairly high frequencies in other experiments. It was subsequently found that indole strongly inhibits the growth of adenine mutants, and it is probably because of this previously unknown fact that no adenineless mutants were obtained. Such an inhibition is not surprising in view of the inhibition of growth of bacteria and yeast by the structurally related benzimidazole, investigated by Wooley.[11]

In a third experiment, ascospores were collected on a medium containing autoclaved fresh liver extract and transferred to tubes containing filter sterilized liver extract. From a total of 145 spores picked, 75 grew when transferred to unheated liver extract medium. One of these cultures failed to grow on minimal medium and has been classified as requiring a heat-labile substance in liver.

Experiments to select for mutants requiring hydrolyzed pantothenic acid or indoleacetic acid were unsuccessful as was an attempt to select a mutant lacking the enzyme urease. In the last case, spores were collected on a medium containing urea as the sole nitrogen source.

Discussion.—It is evident from the experimental data that considerable

progress has been made in developing an improved technique of selecting biochemical mutants in Neurospora. Even with the rigorous discarding of duplicate mutants, 13 per cent of the cultures obtained were found to have special growth requirements. Thus, the present method markedly increases the ease with which such mutants can be obtained.

In the selection of specific mutants three were obtained out of seven attempted. The type requiring adenine probably was not obtained because of inhibition by indole. Since the selection experiments were carried out on a small scale, success in all the selections attempted would be unlikely. Thus, 20 Petri dishes containing a total of 20,000 perithecia would have approximately 300 mutants represented, assuming a mutation frequency of 1.5 per cent. Even if all 300 mutants were represented in the ascopores that were picked, the probability of obtaining a particular mutant that occurred infrequently would not be large. By increasing the number of plates of perithecia, the probability of selecting a particular mutant would, of course, be increased.

It has been previously suspected that some types of mutants have been selected against because of the presence of inhibitors in the "complete" medium used for the isolations. Information obtained in this experimental work suggests that such cases are fairly frequent. At least three mutants have now been found that will not grow on the "complete" medium. These require for growth histidine, tryptophane and methionine, respectively. Thus, it is possible that many groups of mutants have not been obtained because of growth inhibition by substances in the medium used for isolations. It is therefore clear that data on the frequency of occurrence of mutants with different growth requirements should be interpreted with this source of error in mind.

Summary.—1. A new method has been developed for isolating biochemical mutants of Neurospora.

2. The method has been applied successfully to the selection of some specific mutants not previously obtained. The importance of the use of a simple medium for selecting mutants has been discussed.

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[1] Beadle, G. W., *Ann. Rev. Physiol.*, **10**, 17–42 (1948).

[2] Beadle, G. W., *Physiol. Rev.*, **25**, 643–663 (1945).

[3] Horowitz, N. H., *J. Biol. Chem.*, **171**, 255–264 (1947).

[4] Beadle, G. W., and Tatum, E. L., *Am. J. Bot.*, **32**, 678–686 (1945).

[5] Horowitz, N. H., Houlahan, M. B., Hungate, M. G., and Wright, B., *Science*, **104**, 233–234 (1946).

[6] McElroy, W. D., Cushing, J. E., and Miller, H., *J. Cell. Comp. Physiol.*, **30**, 331–346 (1947).

[7] Fries, N., *Nature*, **159**, 199 (1947).
[8] Doermann, A. H., *Arch. Biochem.*, **5**, 373–384 (1944).
[9] Westergaard, M., and Mitchell, H. K., *Am. J. Bot.*, **34**, 573–577 (1947).
[10] Umbreit, W. W., Wood, W. A., and Gunsalus, I. C., *J. Biol. Chem.*, **165**, 731–732 (1946).
[11] Wooley, D. W., *Ibid.*, **152**, 225–232 (1944).

A PREDICTABLE MUTATION IN BACTERIA

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In the bacterium, *Sarcina lutea*, a color mutation has been found which has a number of interesting characteristics. The mutation is stable and does not revert to the original form either spontaneously or under any treatment thus far attempted. It may be produced at any time by either of two different methods of induction, one environmental, the other chemical. It also occurs spontaneously at a low rate.

If the following four species of bacteria, *Bacillus subtilis*, *Proteus vulgaris*, *B. megatherium* and *S. lutea* are grown together in nutrient broth for twenty-four hours and then reisolated by the dilution method, five different strains instead of four are recovered. In addition to the four original species a new non-pigmented form is always obtained. The new type is similar to *S. lutea* in all morphological features except in its color which is white instead of yellow. This new form is here called *S. alba* and is considered to be a mutant form of *S. lutea*.

The above experiment was repeated 15 times and in every case the same mutant form was obtained. About three times as many yellow colonies as white ones are found when the diluted mixture is plated out. All 15 lines of the white mutant have remained stable for at least 50 transfers at two-day intervals while some of the first to be obtained have been cultured for over a year.

Evidence of the stability of the white mutant is as follows. If *S. lutea* and *S. alba* are grown together in nutrient broth and then plated out both white and yellow colonies are recovered. *S. alba* was inoculated into a medium which consisted of an autoclaved forty-eight-hour nutrient broth culture of *S. lutea*. The *S. alba* grew in this medium without any reversion to the yellow form.

Experiments were performed to determine whether a mixture of all four organisms is necessary to produce the white mutation. Each of the original species was left out in turn and mixtures of three species were tested. A

mixture containing *S. lutea* with *B. megatherium* and *P. vulgaris* did not give rise to the white mutant. Mixtures of *S. lutea* with *B. subtilis* and *P. vulgaris* likewise did not result in any mutations. Mixtures of *S. lutea* with *B. subtilis* and *B. megatherium* gave rise to the white mutation but in a much lower proportion of cases, about sixteen yellow to one white colony.

Mixtures of *S. lutea* with one other species of the original three were made but these mixtures of two organisms did not induce the white mutation in any of the three cases.

Another experiment was made in which *S. lutea* was grown in mixed culture with three entirely different species, *Serratia marcescens*, *B. cereus* and *Corynebacterium xerose*. This mixture of four species did not give rise to any mutations.

Observations on the control cultures showed that *S. lutea* in pure culture undergoes spontaneous mutation to *S. alba* at a rate of about one in 40,000 colonies.

Another white mutation, apparently the same or at least indistinguishable from *S. alba*, can likewise be induced at will by growing *S. lutea* in nutrient broth containing various concentrations of nucleic acid (sodium ribose nucleate). After twenty-four hours of incubation such cultures when plated out yield both white and yellow colonies.

Comparison of these observations with other cases of mutation in bacteria will be made in a future paper. Further genetic tests of this mutation and a physiological comparison of *S. lutea* with *S. alba* are in progress.

A CONVEXITY THEOREM

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The theorem which will be proved here has its origin in the study of the following question.

Let $S_n(x)$ denote the partial sum of order n of the Fourier series of the function $g(x) \in L^p$ $(1 < p \leqslant 2)$. Littlewood and Paley[1] have proved that, $n(x)$ being any measurable function taking non-negative integral values, one has the inequality

$$\int_0^{2\pi} \frac{|S_{n(x)}(x)|^p}{\log [n(x) + 2]}\, dx \leqslant A_p \int_0^{2\pi} |g(x)|^p\, dx, \qquad (1)$$

A_p being a constant depending on p only.

The proof of the theorem, when $p \neq 2$,[2] is extremely long and difficult.

The authors of the present paper have long tried to give a proof which would be based on some theorem analogous to Marcel Riesz's convexity theorem. The difficulty lies on the fact that the above inequality does not hold for $p = 1$. If, however, instead of *Fourier series* of the class L^p, we consider *power series* $f(z) = \sum_0^\infty c_\nu z^\nu$ of the Hardy's class H^p, and if we denote by $S_n(\theta)$ the partial sum of order n of the series $f(e^{i\theta}) \sim \sum_0^\infty c_\nu e^{i\nu\theta}$, then the inequality

$$\int_0^{2\pi} \frac{|S_{n(\theta)}(\theta)|^p}{\log [n(\theta) + 2]}\, d\theta \leqslant C_p \int_0^{2\pi} |f(e^{i\theta})|^p\, d\theta \qquad (2)$$

holds for $p = 1$ as well as for $p = 2$. For $p = 2$, the result is equivalent to the Littlewood-Paley result. For $p = 1$, the result is due to Zygmund.[3] Hence, if one could prove some kind of convexity theorem for functions of the class H^p, the inequality (2) would be proved for $1 \leqslant p \leqslant 2$, and (1) would therefore be proved for $1 < p \leqslant 2$, since it is known that the property H^p for a power series is equivalent to the property L^p for the Fourier series which constitutes its real part, provided that $p > 1$.[4]

The purpose of this note is to give a convexity theorem of this kind. In the proof of it, the authors were inspired by a recent paper of Thorin, and especially by his proof of a theorem of Hardy and Littlewood.[5] In his proof, as well as in ours, Thorin's own generalization of M. Riesz's convexity theorem plays an important rôle.[6]

THEOREM. *Let $f(z) = \sum_0^\infty x_\nu z^\nu$ be regular for $|z| < 1$ and belong to the class $H^{1/\alpha}(\alpha > 0)$. Let $M(\alpha, \beta)$ denote the maximum of the finite complex bilinear form*

$$\sum_{j=0}^{m} \sum_{h=0}^{n} a_{jh} x_j y_h$$

when the complex variables x_j, y_h are subject to the conditions

$$\sum_0^n |y_h|^{1/\beta} \leqslant 1 \qquad (\beta \geqslant 0)^7$$

and

$$\int_0^{2\pi} |f(re^{i\theta})|^{1/\alpha} d\theta \leqslant 1 \qquad (\alpha > 0)$$

the second condition holding for every $f(z)$ belonging to $H^{1/\alpha}$ and having x_0, x_1, ... x_m as its $m + 1$ first expansion coefficients, and for every non-negative r inferior to 1. Then, if the point (α, β) lies on the segment joining the points (α_1, β_1) and (α_2, β_2), that is to say if $\alpha = t\alpha_1 + (1 - t)\alpha_2$ and $\beta = t\beta_1 + (1 - t)\beta_2$ $(0 < t < 1)$, we have

$$M(\alpha, \beta) \leqslant C(\alpha_1, \alpha_2)\, M^{t}(\alpha_1, \beta_1)\, M^{1-t}(\alpha_2, \beta_2),$$

the constant $C(\alpha_1, \alpha_2)$ *depending on* α_1 *and* α_2 *only.*

Proof. We start from the assumption that the inequality

$$\Big|\sum_{j=0}^{m}\sum_{h=0}^{n} a_{jh}x_j y_h\Big| \leqslant M(\alpha, \beta)\Big[\int_0^{2\pi}|f(e^{i\theta})|^{1/\alpha}d\theta\Big]^{\alpha}\Big[\sum_0^n |y_h|^{1/\beta}\Big]^{\beta} \quad (3)$$

holds for the points (α_1, β_1) and (α_2, β_2), and for all x_j, y_h.

Let κ be the smallest integer such that $\frac{\kappa}{\alpha_1} \geqslant 2$ and $\frac{\kappa}{\alpha_2} \geqslant 2$, and let $G(z) = \xi_0 + \xi_1 z + \ldots + \xi_p z^p$ be a polynomial of degree p. We now apply the inequality (3) to the case in which $f(z) = G^{\kappa}(z)$.[8] Then x_j, that is to say the coefficient of z^j in $f(z)$, becomes a function $\varphi_j(\xi_0, \xi_1, \ldots \xi_p)$, which is a polynomial, homogeneous, of degree κ, whose coefficients depend on κ and p only. Then (3) becomes

$$\Big|\sum_{j=0}^{m}\sum_{h=0}^{n} a_{jh}\varphi_j(\xi_0, \ldots \xi_p)y_h\Big| \leqslant M(\alpha, \beta)\Big[\int_0^{2\pi}|G(e^{i\theta})|^{\kappa/\alpha}d\theta\Big]^{\alpha}\Big[\sum_0^n |y_h|^{1/\beta}\Big]^{\beta} \quad (4)$$

and is satisfied for the points (α_1, β_1) and (α_2, β_2), and for all values of $\xi_0, \ldots \xi_p$ and $y_0, \ldots y_n$.

Now, by a known theorem on interpolation[9] there exist, for all exponents $q \geqslant 2$, well-determined constants $A_{(q)}$ and $B_{(q)}$ such that

$$A_{(q)}\,\frac{|G_0|^q + |G_1|^q + \ldots + |G_p|^q}{p+1} \leqslant \int_0^{2\pi}|G(e^{i\theta})|^q d\theta \leqslant B_{(q)}\,\frac{|G_0| + \ldots + |G_p|^q}{p+1},$$

where $G_s = G(e^{i\theta_s})$ and $\theta_s = \dfrac{2\pi}{p+1}\, s.$

We now change variables, taking, instead of $\xi_0, \ldots \xi_p$, the variables

$$\eta_s = \xi_0 + \xi_1 e^{i\theta_s} + \ldots + \xi_p e^{ip\theta_s} = G_s$$

as is clearly possible, the determinant being different from zero. The polynomial $\varphi_j(\xi_0, \ldots \xi_p)$ becomes a polynomial $\psi_j(\eta_0, \ldots \eta_p)$, and we get, writing $B = \max.\left\{B\left(\frac{\kappa}{\alpha_1}\right), B\left(\frac{\kappa}{\alpha_2}\right)\right\}$

$$\Big|\sum_{j=0}^{m}\sum_{h=0}^{n} a_{jh}\psi_j(\eta_0, \ldots \eta_p)y_h\Big| \leqslant$$

$$M(\alpha, \beta)\Big[B\,\frac{|\eta_0|^{\kappa/\alpha} + \ldots + |\eta_p|^{\kappa/\alpha}}{p+1}\Big]^{\alpha}\Big[\sum_0^n |y_h|^{1/\beta}\Big]^{\beta}$$

this inequality being valid for the points (α_1, β_1) and (α_2, β_2) and for all $\eta_0, \ldots \eta_p$ and $y_0, \ldots y_n$.

We apply now Thorin's generalization of Marcel Riesz's convexity theorem, and we get, writing A instead of $A\left(\frac{\kappa}{\alpha}\right)$,

$$\left|\sum_{j=0}^{m}\sum_{k=0}^{n} a_{jk}\psi_j(\eta_0 \ldots \eta_p)y_k\right| \leqslant$$

$$\left[M(\alpha_1, \beta_1)\frac{B^{\alpha_1}}{(p+1)^{\alpha_1}}\right]^{t}\left[M(\alpha_2, \beta_2)\frac{B^{\alpha_2}}{(p+1)^{\alpha_2}}\right]^{1-t}\left[\sum_0^p|\eta_s|^{\kappa/\alpha}\right]^{\alpha}\left[\sum_0^n|y_k|^{1/\beta}\right]^{\beta} \leqslant$$

$$M^t(\alpha_1, \beta_1)\, M^{1-t}(\alpha_2, \beta_2)\left[\frac{B}{p+1}\sum_0^p|\eta_s|^{\kappa/\alpha}\right]^{\alpha}\left[\sum_0^n|y_k|^{1/\beta}\right]^{\beta} \leqslant$$

$$\left(\frac{B}{A}\right)^{\alpha} M^t(\alpha_1, \beta_1)\, M^{1-t}(\alpha_2, \beta_2)\left[\int_0^{2\pi}|G(e^{i\theta})|^{\kappa/\alpha}d\theta\right]^{\alpha}\left[\sum_0^n|y_k|^{1/\beta}\right]^{\beta}$$

Thus also

$$\left|\sum_{j=0}^{m}\sum_{k=0}^{n} a_{jk}x_jy_k\right| \leqslant$$

$$\left(\frac{B}{A}\right)^{\alpha} M^t(\alpha_1, \beta_1)M^{1-t}(\alpha_2, \beta_2)\left[\int_0^{2\pi}|f(e^{i\theta})|^{1/\alpha}d\theta\right]^{\alpha}\left[\sum_0^n|y_k|^{1/\beta}\right]^{\beta}$$

where $x_0, x_1, \ldots x_m$ are the first $m+1$ coefficients of $f(z) = G^{\kappa}(z)$, $G(z)$ being a polynomial and κ being fixed as said before.

The last inequality being true when G is any polynomial, it remains true when G is any function of the class $H^{\kappa/\alpha}$. Hence it is also true when $f(z)$ is any function of the class $H^{1/\alpha}$ and having no zeros for $|z| < 1$, for then we can always write $f(z) = G^{\kappa}(z)$, G being regular.

From this we deduce, since any function $f(z)$ of the class $H^{1/\alpha}$ is the sum of two "zero-free" functions $f_1(z)$ and $f_2(z)$ of the same class, with $|f_1| \leqslant 2|f|$ and $|f_2| \leqslant 2|f|$, that, D being an absolute constant,

$$\left|\sum_{j=0}^{m}\sum_{k=0}^{n} a_{jk}x_jy_k\right| \leqslant$$

$$\left(\frac{B}{A}\right)^{\alpha} DM^t(\alpha_1, \beta_1)\, M^{1-t}(\alpha_2, \beta_2)\left[\int_0^{2\pi}|f(e^{i\theta})|^{1/\alpha}d\theta\right]^{\alpha}\left[\sum_0^n|y_k|^{1/\beta}\right]^{\beta}$$

for any function $f \in H^{1/\alpha}$ regular for $|z| < 1$. This gives just our theorem, if we observe that $\alpha_1 < \alpha < \alpha_2$, that κ and B depend only on α_1 and α_2, and that the minimum of $A = A\left(\frac{\kappa}{\alpha}\right)$, when $\frac{\kappa}{\alpha}$ lies between $\frac{\kappa}{\alpha_1}$ and $\frac{\kappa}{\alpha_2}$, depends also on α_1 and α_2 only.

Writing $X_h = \sum_{j=0}^{m} a_{jh}x_j$ $(h = 0, 1, \ldots n)$, it is familiar that $M(\alpha, \beta)$ is also the maximum of the ratio $(\sum_0^n |X_h|^{1/\beta'})^{\beta'}/[\int_0^{2\pi}|f(e^{i\theta})|^{1/\alpha}]^{\alpha}$, where $\beta + \beta' = 1$. From sums of powers of linear forms we pass, by the classical process, to integrals, and the theorem of Littlewood and Paley is the consequence of the inequality (2) applied to the two extreme cases $\alpha = \beta' = {}^1/_2$ $(p = 2)$ and $\alpha = \beta' = 1$ $(p = 1)$.

Remark: In our main theorem, the condition on the y's can be replaced by one similar to that imposed on the x's. But this has no interesting application. By passages to limits we can also obtain results about linear operations from H^p to L^q, where $p, q \geqslant 1$.

[1] Littlewood and Paley, *Proc. London Math. Soc.*, **43**, 105 (1937).

[2] For a simple proof in the case of $p = 2$, see Hardy and Littlewood, *Proc. Cambridge Phil. Soc.*, **40**, 103–107 (1938).

[3] See Zygmund, A., *Fundamenta Mathematicae*, **30**, 190 (1938).

[4] A function $f(z) = \Sigma c_n z^n$, regular for $|z| < 1$, is said to belong to the class H^p if $\int_0^{2\pi}|f(re^{i\theta})|^p d\theta$ remains bounded as $r \to 1$. For the classical results of the theory see, e.g., Zygmund, *Trigonometrical Series*, Chapter VII.

[5] Thorin, G. O., *Convexity Theorems*, Uppsala, 1948, pp. 1–57, esp. 31–35.

[6] Thorin, G. O., "An Extension of a Convexity Theorem Due to M. Riesz," *Kungl. Fysiografiska Sällskapets i Lund Forhändliger*, 8 (1939), nr. 14. Tamarkin, J. D., and Zygmund, A., "Proof of a Theorem of Thorin," *Bull. the Am. Math. Soc.*, **50**, 279–282 (1944). Salem, R., Sur une extension du théorème de convexité de M. Marcel Riesz, *Colloquium Mathematicum*, Wroclaw, 1947, Vol. I, pp. 6–8.

[7] In the case $\beta = 0$, the condition is to be interpreted, as usual, as max. $|y_h| \leq 1$.

[8] Thorin's idea (see footnote 5) of taking for f the kth power of an analytic function and applying his convexity theorem, is basic for the whole argument. If we wanted to restrict ourselves to the proof of the Littlewood-Paley result, the argument could be simplified still further, since here we interpolate between $\alpha = 1$ and $\alpha = {}^1/_2$, and we could imitate more closely the proof of Thorin (see footnote 5, pp. 31–35).

[9] See *e.g.* Marcinkiewicz and Zygmund, *Fundamenta Mathematicae*, **28**, 131–166 (1937).

NOTES ON INTEGRATION, II

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The results of our first note[1] enable us to treat the following classes of functions, all contained in $\mathfrak{G}$:

$$\mathfrak{F}_p = \{f;\ f|f|^{p-1} \in \mathfrak{F}\} = \{f;\ N(|f|^p) < +\infty\}, \qquad \mathfrak{L}_p = \{f;\ f|f|^{p-1} \in \mathfrak{L}\},$$
$$\mathfrak{M} = \{f;\ \text{mid}\,(f, g, h) \in \mathfrak{L} \textit{ for all } g \textit{ and } h \textit{ in } \mathfrak{L}\},$$

where $p \geqq 1$ and mid (λ, μ, ν) designates the intermediate one of the three numbers λ, μ, ν in accordance with the precise relations

$$\begin{aligned} \text{mid } (\lambda, \mu, \nu) &= \max (\min (\lambda, \mu), \min (\mu, \nu), \min (\nu, \lambda)) \\ &= \min (\max (\lambda, \mu), \max (\mu, \nu), \max (\nu, \lambda)). \end{aligned}$$

Obviously, $\mathfrak{F}_1 = \mathfrak{F}$ and $\mathfrak{L}_1 = \mathfrak{L}$. The importance of the class $\mathfrak{L}_p$ is well established, and the consideration of $\mathfrak{F}_p$ along with $\mathfrak{L}_p$ is natural. Since in the classical instances of our theory $\mathfrak{M}$ can be identified as the totality of measurable functions, we shall call any function in $\mathfrak{M}$ a *measurable function*.

In order to discuss $\mathfrak{F}_p$ we need to establish for the quantity $N_p(f) = N(|f|^p)^{1/p}$ the inequalities

(1) (Hölder) *if* $p > 1$ *and* $p + q = pq$, *then* $N(fg) \leqq N_p(f)N_q(g)$;
(2) (Minkowski) $N_p(f + g) \leqq N_p(f) + N_p(g)$.

The proof of (1) begins with the observation that for $\alpha > 0$, $\beta > 0$, and $\gamma = 1/[(q/p)^{1/q} + (p/q)^{1/p}]$ the function $\gamma(\alpha\xi^p + \beta\xi^{-q})$, $0 < \xi < +\infty$, has $\alpha^{1/p}\beta^{1/q}$ as its absolute minimum and assumes this value only when $\xi = \xi_0 = (\beta q/\alpha p)^{1/pq}$. As a result we see that $|fg| \leqq \gamma(|f|^p\xi^p + |g|^q\xi^{-q})$ and hence that $N(fg) \leqq \gamma(N(|f|^p)\xi^p + N(|g|^q)\xi^{-q})$ for all $\xi > 0$. On putting $N(|f|^p) = \alpha$, $N(|g|^q) = \beta$, and $\xi = \xi_0$ in the latter inequality we obtain (1). Since for $p = 1$ the inequality (2) has already been established (as a special case of I (7)), we suppose that $p > 1$. The proof for this case then begins with the observation that $|\xi + \eta|^p \leqq 2^{p-1}(|\xi|^p + |\eta|^p)$. We therefore have $N(|f + g|^p) \leqq 2^{p-1}(N(|f|^p) + N(|g|^p))$, so that $f + g$ is in $\mathfrak{F}_p$ whenever f and g are. Consequently we can use (1) and the relation $|f + g|^p \leqq |f|\,|f + g|^{p-1} + |g|\,|f + g|^{p-1}$ to obtain

$$\begin{aligned} N(|f + g|^p) &\leqq N(|f|\,|f + g|^{p-1}) + N(|g|\,|f + g|^{p-1}) \\ &\leqq (N_p(f) + N_p(g))N(|f + g|^p)^{1/q} \end{aligned}$$

an inequality from which (2) follows at once. The Minkowski inequality shows immediately that identification of functions f and g for which $N_p(f - g) = 0$ will permit us to treat $\mathfrak{F}_p$ as a real normed vector-lattice with N_p as its norm-function. It is easily verified that this identification is the same for all values of p, two functions being identified if and only if they are equal almost everywhere. We can now generalize I (10) to read

(3) *the normed vector space* $\mathfrak{F}_p$ *is complete* (*and hence a Banach space*).

The proof will be sketched for $p > 1$. By definition the mapping Φ which carries f into $g = |f|\,|f|^{p-1} = |f|^p \operatorname{sgn} f$ maps $\mathfrak{F}_p$ onto $\mathfrak{F}$. It has as its inverse the mapping Ψ which carries g into $f = |g|^{1/p} \operatorname{sgn} g$. The local

behavior of these mappings can be determined by an appeal to the inequalities[2]

$$2^{1-p}|\xi - \eta|^p \leqq |\xi|\xi|^{p-1} - \eta|\eta|^{p-1}| \leqq p|\xi - \eta|(|\xi|^{p-1} + |\eta|^{p-1}),$$

which hold even for complex ξ and η. The first yields $|\Psi(g_1) - \Psi(g_2)|^p \leqq 2^{p-1}|g_1 - g_2|$ and hence $N_p(\Psi(g_1) - \Psi(g_2)) \leqq 2^{1/q}N(g_1 - g_2)^{1/p}$ for all g_1 and g_2 in $\mathfrak{F}$. Accordingly Ψ is continuous, belonging in fact to the Lipschitz class Lip $1/p$. Similarly, the second inequality yields $|\Phi(f_1) - \Phi(f_2)| \leqq p(|f_1 - f_2||f_1|^{p-1} + |f_1 - f_2||f_2|^{p-1})$ and hence, with the help of the Hölder inequality,

$$N(\Phi(f_1) - \Phi(f_2)) \leqq pN_p(f_1 - f_2)(N_p(f_1)^{p/q} + N_p(f_2)^{p/q}).$$

Accordingly Φ is continuous, belonging *on any bounded part of* $\mathfrak{F}_p$ to the associated Lipschitz class Lip 1. If now $\{f_n\}$ is a Cauchy sequence in $\mathfrak{F}_p$, it is bounded and must therefore be carried by Φ into a Cauchy sequence $\{g_n\}$, $g_n = \Phi(f_n)$, in $\mathfrak{F}$. The completeness of $\mathfrak{F}$ shows that the latter sequence has a limit g in $\mathfrak{F}$. Thus the function $f = \Psi(g)$ is the limit of $\{f_n\}$ in $\mathfrak{F}_p$, by virtue of the continuity of Ψ. This completes the proof. We note further that we have obtained at the same time the following variant of a result of Mazur:[2]

(4) *The spaces* $\mathfrak{F}_p$, $p \geqq 1$, *are mutually homeomorphic and all have the same linear dimension.*

The first part of (4) follows from the fact that Φ maps $\mathfrak{F}_p$ homeomorphically onto $\mathfrak{F}$, as shown above. The second results from a topological interpretation of the linear dimension. It is well known that a Banach space has finite linear dimension if and only if it is locally compact, in which case it is homeomorphic to an n-dimensional Euclidean space and its linear dimension is n. On the other hand, if the linear dimension of a Banach space is infinite it is equal to the density-character of the space—that is, to the least among the cardinal numbers of everywhere dense parts of the space.

Turning now to the consideration of $\mathfrak{L}_p$, we proceed to specialize and sharpen the results of the previous paragraph. First we replace (1) by the more detailed statement

(1′) (Hölder) *if* $f \in \mathfrak{L}_p$ *and* $g \in \mathfrak{L}_q$, *then* $fg \in \mathfrak{L}$ *and* $|L(fg)| \leqq L(|f|^p)^{1/p} \cdot L(|g|^q)^{1/q}$, *the equality holding if and only if* $f|f|^{p-1}$ *and* $g|g|^{q-1}$ *are linearly dependent in* $\mathfrak{L}$ (*when* $p = q = 2$, *if and only if* f *and* g *are linearly dependent in* $\mathfrak{L}_2$).

To show that $fg \in \mathfrak{L}$ under the present hypotheses, we appeal to I (13), writing $fg = \varphi(f', g')$ where $f' = f|f|^{p-1} \in \mathfrak{L}$, $g' = g|g|^{q-1} \in \mathfrak{L}$, and $\varphi(\lambda, \mu) = |\lambda|^{1/p}|\mu|^{1/q}$ sgn $\lambda\mu$. Thus we have

$$|L(fg)| \leqq L(|fg|) = N(fg) \leqq N_p(f)N_q(g) = L(|f|^p)^{1/p}L(|g|^q)^{1/q}$$

by virtue of (1). In determinlng the conditions under which the extreme terms here are equal, we may discard the trivial case where the last term vanishes, one of the two functions f and g being a null function. The indicated equality is equivalent to the continued equation $\pm L(fg) = L(|fg|) = L(|f|^p)\xi_0^p + L(|g|^q)\xi_0^{-q} = L(|f|^p\xi_0^p + |g|^q\xi_0^{-q})$, where ξ_0 has the value indicated in the proof of (1) and a fixed determination of the ambiguous sign is adopted for the remainder of the discussion. In view of the relations $\pm fg \leqq |fg| \leqq |f|^p\xi_0^p + |g|^q\xi_0^{-q}$, the continued equation for the integrals holds if and only if $\pm fg = |fg| = |f|^p\xi_0^p + |g|^q\xi_0^{-q}$ almost everywhere. The second equation here holds if and only if $|g|^q = (p/q)\xi_0^{pq}|f|^p$ almost everywhere. Consequently both equations hold if and only if $g|g|^{q-1} = \pm(p/q)\xi_0^{pq}f|f|^{p-1}$ almost everywhere. The proof of (1′) is thereby completed. In a quite similar fashion we find that (2) can be replaced by

(2′) (Minkowski) *if f and g are in $\mathfrak{L}_p$, then $f + g$ is in $\mathfrak{L}_p$ and $L(|f + g|^p)^{1/p} \leqq L(|f|^p)^{1/p} + L(|g|^p)^{1/p}$ the equality holding if and only if f and g are linearly dependent in $\mathfrak{L}_p$ and of the same sign almost everywhere.*

To show that $f + g \in \mathfrak{L}_p$ under the present hypotheses, we appeal to I (13), writing $(f + g)|f + g|^{p-1} = \varphi(f', g')$ where $f' = f|f|^{p-1} \in \mathfrak{L}, g' = g|g|^{p-1} \in \mathfrak{L}$, and $\varphi(\lambda, \mu) = (|\lambda|^{1/p} \operatorname{sgn} \lambda + |\mu|^{1/p} \operatorname{sgn} \mu)||\lambda|^{1/p} \operatorname{sgn} \lambda + |\mu|^{1/p} \operatorname{sgn} \mu|^{p-1}$. The result given in (2) now assumes the present form. Reviewing the proof of (2) under the conditions postulated here we see that the equality holds if and only if $|f + g| = |f| + |g|$ almost everywhere while $|f|^p$, $|g|^p$, and $|f + g|^p$ are linearly dependent in $\mathfrak{L}$ in harmony with (1′); but these conditions are equivalent to those stated above. Since $\mathfrak{L}_p$ clearly contains αf and $|f|$ together with f, it is a linear sublattice of $\mathfrak{F}_p$. Moreover the fact that the homeomorphic mapping Φ carries $\mathfrak{L}_p$ onto $\mathfrak{L}$, where $\mathfrak{L}$ is closed in $\mathfrak{F}$, shows that $\mathfrak{L}_p$ is closed in $\mathfrak{F}_p$. Accordingly we have

(3′) *$\mathfrak{L}_p$ is a closed linear subspace of $\mathfrak{F}_p$ (and hence is a Banach space).*

We can give a rather wide extension of (4) which will cover not only the spaces $\mathfrak{L}_p$, $p \geqq 1$, arising from a fixed elementary integral but also the spaces $\mathfrak{L}_p$ arising from different elementary integrals. This is possible because the space $\mathfrak{L}_2$ is a generalized Euclidean or real Hilbert space, its norm being obtained in the appropriate manner from the integral $L(fg)$, which depends linearly and symmetrically upon f and g. Two such spaces are homeomorphic if and only if they have the same linear dimension, as is well known. Hence we have

(4′) *the spaces* $\mathfrak{L}_p$, $p \geqq 1$, *arising from a fixed elementary integral are mutually homeomorphic and all have the same linear dimension; two such spaces arising from different elementary integrals are homeomorphic if and only if they have the same linear dimension.*

This result completes our discussion of the spaces $\mathfrak{L}_p$.

In the study[3] of the class $\mathfrak{M}$, it is convenient to observe that mid (λ, μ, ν) is a positively homogeneous, continuous function satisfying the inequalities min $(\mu, \nu) \leqq$ mid $(\lambda, \mu, \nu) \leqq$ max (μ, ν), since we thereby justify applications of I (13) and of the dominated-convergence theorem at various points below. It is, of course, true that mid (λ, μ, ν) has an explicit expression in terms of λ, μ, ν by means of addition, multiplication by constants and formation of absolute values. Thus we can show at once that

(5) *if* $f = \lim_{n\to\infty} f_n$ *and* $f_n \in \mathfrak{M}$, *then* $f \in \mathfrak{M}$.

In fact we have mid $(f_n, g, h) \in \mathfrak{L}$ whenever g and h are in $\mathfrak{L}$, and hence conclude by the dominated-convergence theorem that mid $(f, g, h) = \lim_{n\to\infty}$ mid (f_n, g, h) is also in $\mathfrak{L}$. We also have

(6) *if* $f_1, \ldots, f_m$ *are in* $\mathfrak{M}$ *and if* $\varphi(\lambda_1, \ldots, \lambda_m)$ *is any positively homogeneous function of Baire (bounded or not) defined for* $-\infty \leqq \lambda_k \leqq +\infty$ *then* $\varphi(f_1, \ldots, f_m) \in \mathfrak{M}$; *in particular, the functions* αf, $|f|$, $f + g$ *are measurable whenever* f *and* g *are.*

Once the indicated particular cases have been handled, the general case is obtained by passages to the limit similar to those used in deriving I (13) from I (11). Taking the case of $|f|$ as typical, we wish to show that mid $(|f|, g, h) \in \mathfrak{L}$ whenever g and h are in $\mathfrak{L}$. By hypothesis the function $f_n =$ mid $(f, n(|g| + |h|), -n(|g| + |h|))$ is in $\mathfrak{L}$; and so also is the function $g_n =$ mid $(|f_n|, g, h)$. It is easily seen that $\lim_{n\to\infty} f_n(x)$ has the value 0 or the value $f(x)$ according as $|g(x)| + |h(x)|$ vanishes or not. Hence $\lim_{n\to\infty} g_n =$ mid $(|f|, g, h)$, and the latter function is in $\mathfrak{L}$ by the dominated-convergence theorem. In practice it is convenient to have available the following criterion:

(7) *when* $f \geqq 0$, *a necessary and sufficient condition for* f *to be measurable is that* min $(f, g) \in \mathfrak{L}$ *whenever* $g \in \mathfrak{L}$ *and* $g \geqq 0$.

The necessity of the stated condition results immediately from the identity min $(f, g) =$ mid $(f, g, 0)$. The sufficiency is established by noting that for arbitrary g and h in $\mathfrak{L}$ we have mid $(f, g, h) =$ max (min $(f,$ max $(g, h))$, min $(g, h)) \in \mathfrak{L}$ since min $(f,$ max $(g, h)) =$ min $(f,$ max $(g, h,$

0)) + min (0, max $(g, h)) \in \mathfrak{L}$ by hypothesis. From (7) and I (14) we have

(8) *the constant function* 1 *is measurable if and only if min* $(1, g)$ *is integrable whenever* g *is; and it is measurable whenever* I (3) *is verified.*

Hence we immediately obtain the following generalization of (6) above

(9) *if* $1, f_1, \ldots, f_m$ *are measurable and if* $\varphi(\lambda_1, \ldots, \lambda_m)$ *is any function of Baire (bounded or not) defined for* $-\infty \leqq \lambda_k \leqq +\infty$, *then* $\varphi(f_1, \ldots, f_m) \in \mathfrak{M}$.

We conclude our general remarks on measurable functions by noting some criteria for integrability. First we have

(10) *in order that a measurable function* f *be integrable it is necessary and sufficient that* $N(f) < +\infty$; *and it is likewise necessary and sufficient there exist integrable functions* g *and* h, *such that* $g \leqq f \leqq h$.

If f is integrable, then $N(f) < +\infty$ and the second condition is satisfied with $g = h = f$. If $N(f) < +\infty$ we can find a function $k \in \mathfrak{L}$ such that $|f| \leqq k$, since there exist elementary functions f_n such that $|f| \leqq \sum_{n=1}^{\infty} |f_n|$, $\sum_{n=1}^{\infty} E(|f_n|) < +\infty$, and the function $k = \sum_{n=1}^{\infty} |f_n|$ is in $\mathfrak{L}$ by virtue of I (12). Thus $N(f) < +\infty$ implies that $g \leqq f \leqq h$ where $g = -k$ and $h = k$ are in $\mathfrak{L}$. When there exist such g and h, we have $f = \text{mid } (f, g, h) \in \mathfrak{L}$ by the definition of $\mathfrak{M}$. Since any integrable function is clearly measurable, by direct application of the definition, we see that (10) yields the relations

$$(11) \quad \mathfrak{L} = \mathfrak{M} \cap \mathfrak{F} = \{f;\ f \in \mathfrak{M},\ N(f) < +\infty\}.$$

It follows immediately that

$$(12) \quad \mathfrak{L}_p = \{f;\ f|f|^{p-1} \in \mathfrak{M},\ N_p(f) < +\infty\}.$$

When 1 is measurable we can give the last result the sharper form,

$$(13) \quad \text{if } 1 \in \mathfrak{M}, \text{ then } \mathfrak{L}_p = \mathfrak{M} \cap \mathfrak{F}_p = \{f;\ f \in \mathfrak{M},\ N_p(f) < +\infty\},$$

since (9) shows that the functions $g = f|f|^{p-1}$ and $f = |g|^{1/p} \operatorname{sgn} g$ are measurable together.

Finally we shall consider the connections between our theory of the general integral and the theory of measure. By specializing I (5)–(7) we see that an outer measure μ^* is defined for the subsets of X by putting $\mu^*(Y) = N(f_Y)$ where f_Y is the characteristic function of $Y \subset X$. Similarly, we see from the properties of the general integral that the finite set-function μ defined by putting $\mu(Y) = L(f_Y)$ whenever $f_Y \in \mathfrak{L}$ is a *completely additive*

measure. It is natural to define a set Y to be measurable if and only if $f_Y \in \mathfrak{M}$; but we must then consider how such sets are related to the μ^*-*measurable sets* Y, characterized by the now classical condition that $\mu^*(Z) = \mu^*(Z \cap Y) + \mu^*(Z \cap Y')$ for all Z. A fundamental tool in this investigation is provided by the following result:

(14) *if* $N(f) < +\infty$, *there exists a function* $g \in \mathfrak{L}$ *such that* $|f| \leqq g$ *and* $N(f) = L(g)$—*in particular, if* $1 \in \mathfrak{M}$ *and* f *is a characteristic function, then* g *may be chosen to be a characteristic function.*

Adopting a device used in the proof of (10) above, we select elementary functions f_{mn} such that $|f| \leqq \sum_{n=1}^{\infty} |f_{mn}| = g_m$, $N(f) \leqq \sum_{n=1}^{\infty} E(|f_{mn}|) \leqq N(f) + 1/m$ and note that $g_m \in \mathfrak{L}$ and $N(f) \leqq L(g_m) \leqq N(f) + 1/m$ in accordance with I (12). It is then clear that $g = \lim_{m\to\infty} \min(g_1, \ldots, g_m)$ has the desired properties. In the indicated special case, we may first suppose that $g \leqq 1$ since otherwise we can replace g by $\min(1, g) \in \mathfrak{L}$ in accordance with (8); and we may then suppose that g is a characteristic function, since otherwise we can replace g by the characteristic function $\lim_{n\to\infty} g^n \in \mathfrak{L}$, in accordance with (9), (10) and the dominated-convergence theorem. From (14) we obtain

(15) *every measurable set is* μ^*-*measurable; but the converse is true if and only if* $1 \in \mathfrak{M}$.

We sketch the proof. Assuming Y to be measurable, we have to reduce the inequality $\mu^*(Z) \leqq \mu^*(Z \cap Y) + \mu^*(Z \cap Y')$ to an equality. Since the reduction is automatic when $\mu^*(Z) = +\infty$, we suppose that $\mu^*(Z) < +\infty$. Then (14) furnishes a function $g \in \mathfrak{L}$ such that $f_Z \leqq g$, $\mu^*(f_Z) = L(g)$. Since $f_{Z\cap Y} = f_Z f_Y \leqq g f_Y = \min(g, f_Y) \leqq g$ we see that $g f_Y \in \mathfrak{L}$, $\mu^*(Z \cap Y) \leqq L(g f_Y)$. The inequality $f_{Z\cap Y'} = f_Z(1 - f_Y) \leqq g(1 - f_Y) = g - g f_Y \in \mathfrak{L}$ shows that $\mu^*(Z \cap Y') \leqq L(g - g f_Y)$. By addition we obtain $\mu^*(Z \cap Y) + \mu^*(Z \cap Y') \leqq L(g f_Y) + L(g - g f_Y) = L(g) = \mu^*(Z)$, thereby completing the discussion. Looking now at the converse, we see that X is trivially μ^*-measurable and hence that the converse cannot hold unless $1 = f_X \in \mathfrak{M}$. Assuming this necessary condition, we now show that $f_Y \in \mathfrak{M}$ by (7), whenever Y is μ^*-measurable. Starting with an arbitrary non-negative integrable function g, we put $f_n = \varphi_n(g) \leqq ng$, $g_n = g f_n \leqq g$ where φ_n is the characteristic function of the interval $1/n \leqq \lambda \leqq +\infty$. By (9) and (10) we see that f_n and g_n are integrable. Moreover f_n is the characteristic function of a set Z_n with $\mu(Z_n) = L(f_n)$. Since $\min(f_Y, g) = \lim_{n\to\infty} \min(f_Y, g_n) = \lim_{n\to\infty} \min(f_Y f_n, g) \leqq g$ it suffices to show that $f_Y f_n \in \mathfrak{L}$. Since $f_Y f_n$ is the characteristic function of $Y \cap Z_n$, the special

case of (14) furnishes us with a set W_n such that $Y \cap Z_n \subset W_n$, $\mu^*(Y \cap Z_n) = \mu(W_n)$. We may suppose that $W_n \subset Z_n$, since otherwise we can replace it by $W_n \cap Z_n$. We now have $\mu(W_n) = \mu^*(W_n) = \mu^*(W_n \cap Y) + \mu^*(W_n \cap Y') \geqq \mu^*(W_n \cap Y) = \mu^*(Y \cap Z_n) = \mu(W_n)$. Hence $\mu^*(W_n \cap Y') = 0$ and $W_n \cap Y'$ is a null set. Thus $f_Y f_n$ differs from the characteristic function of W_n by a null function and is integrable. This completes the proof. From (14) and (15) we at once derive

(16) *when* $1 \in \mathfrak{M}$, $\mu^*(Y) < +\infty$ *is the minimum of the measures* $\mu(Z)$ *where* $Z \supset Y$; *and* $\mu(Y)$ *exists if and only if* Y *is* μ^**-measurable and has finite outer measure, in which case* $\mu(Y) = \mu^*(Y)$.

On the basis of the preceding results, we can now establish

(17) *when* $1 \in \mathfrak{M}$, *a finite function* f *is measurable if and only if the sets* $\{x;\ \alpha \leqq f(x) < \beta\}$ *are measurable for all (rational)* α *and* β, $\alpha > \beta$; *and, when* f *is integrable, its general integral* $L(f)$ *is the limit of the Lebesgue sums*

$$\sigma(f;\ \epsilon) = \sum_{k=-\infty}^{k=+\infty}{}' \sigma_k \mu\{x;\ \alpha_k \leqq f(x) < \alpha_{k+1}\},$$

where $\lim_{k \to -\infty} \alpha_k = -\infty$, $\lim_{k \to +\infty} \alpha_k = +\infty$, $\alpha_k \leqq \sigma_k \leqq \alpha_{k+1}$, $\alpha_{k+1} - \alpha_k \leqq \epsilon$, *and* $\sigma_k = 0$ *for min* $(|\alpha_k|, |\alpha_{k+1}|) < \epsilon$, *the summation omitting those terms in which* $\sigma_k = 0$.

The measurability of the set $\{x;\ \alpha \leqq f(x) < \beta\}$ is equivalent to that of its characteristic function, expressible as $\varphi_{\alpha\beta}(f)$ where $\varphi_{\alpha\beta}$ is the characteristic function of the interval $\alpha \leqq \lambda < \beta$. By (9) this function is measurable when f is. On the other hand, if $\varphi_{\alpha\beta}(f)$ is measurable for all α and β (or even just for rational α and β), we see that the function $f_\epsilon = \sum_{k=-\infty}^{k=+\infty} \sigma_k \varphi_k(f)$, where $\varphi_k = \varphi_{\alpha_k \alpha_{k+1}}$, is measurable (for rational α_k, at least) and that its limit $f = \lim_{\epsilon \to 0} f_\epsilon$ is also, in accordance with (5) and (6). When f is integrable and $\gamma_k = \min(|\alpha_k|, |\alpha_{k+1}|) \geqq \epsilon$, we see that $\varphi_k(f) \leqq |f|/\gamma_k$, $|f_\epsilon| \leqq 2\sum_{k=-\infty}^{k=+\infty}{}' \gamma_k \varphi_k(f) \leqq 2|f|$ by virtue of the inequality $|\sigma_k| \leqq \min(|\alpha_k|, |\alpha_{k+1}|) + \epsilon \leqq 2\gamma_k$; and hence that $\varphi_k(f)$ and f_ϵ are both integrable in accordance with (10). The dominated-convergence theorem then yields $L(f) = \lim_{\epsilon \to 0} L(f_\epsilon)$, where $L(f_\epsilon) = \sum_{k=-\infty}^{k=+\infty}{}' \sigma_k L(\varphi_k(f)) = \sigma(f;\ \epsilon)$, as we wished to show. If we apply these considerations to a particular instance of our general theory we obtain immediately an important theorem:[4]

(18) *if* E *is any positive linear functional on the real vector-lattice* $\mathfrak{L}$ *of*

all continuous real functions with compact nucleus on a locally compact space X, then E can be expressed as an integral in the sense of Lebesgue with respect to a measure on X.

As we have already pointed out, $\mathfrak{E}$ and E satisfy our basic postulates and therefore lead to the introduction of an associated general integral and an associated outer measure. Since I (3) is valid here, we see that $1 \epsilon \mathfrak{M}$ and hence that (15), (16) and (17) are valid also.

It is an open question to determine what modifications (if any) in our definitions and procedures will permit a more thorough analysis of the case where 1 is not measurable.

[1] Stone, M. H., "Notes on Integration, I," these PROCEEDINGS, **34**, 336–342 (1948); cited as I.

[2] Mazur, S., "Une remarque sur l'homéomorphie des champs fonctionnels," *Studia Math.*, **1**, 83–85 (1929).

[3] Mr. H. Rubin, while a member of one of my classes, worked out much useful information on the subject of this paragraph and the next.

[4] This result is closely related to theorems given by Riesz, F., "Sur certains systèmes singuliers d'équations intégrales," *Ann. Sci. de l'Ec. Norm. Sup.* (3), **28**, 33–62 (1911); Markoff, A., "On Mean Values and Exterior Densities," *Mat. Sbornik*, **4**, 165–191 (1938), especially Theorems 17 and 20; Kakutani, S., "Concrete Representation of Abstract M-Space," *Ann. Math.*, **42**, 994–1024 (1941), especially Theorem 9. The measure found in (18) is always regular in the sense of Carathéodory; but it is defined with certainty only for compact G_δ-sets and not necessarily for all compact sets (except, of course, when X is separable). This measure is therefore not necessarily identical with the one introduced by Markoff. The distinction is that between "Baire measures" and "Borel measures" (in the terminology of P. R. Halmos) and is known to be genuine on the basis of an unpublished example of J. Dieudonné. We shall have more to say about this situation in our fourth note.

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*SOME PRELIMINARY RESULTS ON THE SPECTRA OF AsH_3, AsD_3 AND PH_3**

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Measurements have been made on several of the bands in the spectra of AsH_3, AsD_3 and PH_3. Work is progressing on the analysis of these bands, but since some considerable time will be required to bring this work to completion we wish here to present a report on the work accomplished thus far on the spectra of these molecules.

Figure 1 represents the 4.5 μ absorption region in the spectrum of AsH_3 gas. This is the region studied earlier by Lee and Wu[1] under a resolving

power somewhat smaller than ours. We believe this region to consist of the two fundamental bands ω_1 and ω_2 (using the notation of Dennison) where ω_1 is a vibration where the electric moment oscillates along the axis of symmetry and ω_2 is a twofold degenerate oscillation normal to the axis of symmetry. The sharp set of lines is taken to be those associated

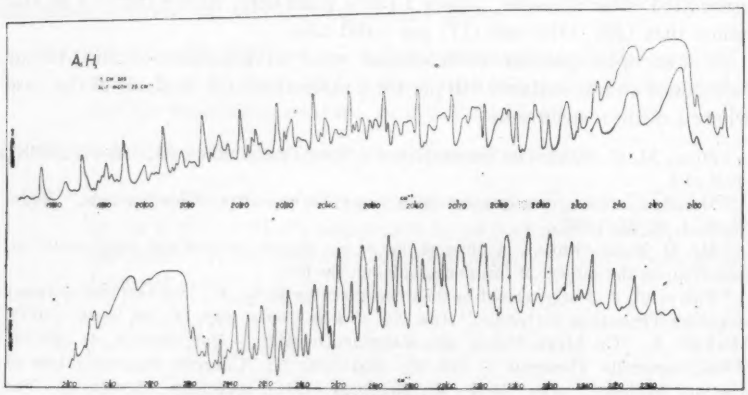

FIGURE 1

ω_1 and ω_2 in the infra-red spectrum of AsH_3.

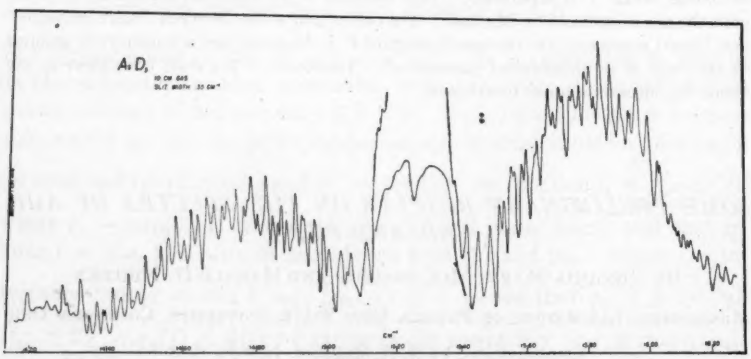

FIGURE 2

ω_1 and ω_2 in the infra-red spectrum of AsD_3.

with ω_1 while the diffuse set (particularly so on the low-frequency side) are taken to be associated with ω_2. An analysis of ω_1 has led to the values $B'' = 3.723$ cm.$^{-1}$ and $B' = 3.682$ cm.$^{-1}$, where the B's are the reciprocals of inertia, $h/8\pi^2cI_{zz}$, effective in the normal state and the vibration state ω_1. The band center ν_1 is found to have the value 2116.1 cm.$^{-1}$

Figure 2 represents the corresponding region in the spectrum of heavy arsine. An analysis of ω_1 in this spectrum leads to the values $B'' = 1.896$ cm.$^{-1}$ and $B' = 1.883$ cm.$^{-1}$ for the reciprocals of inertia of this molecule in its normal and excited states. The band center ν_1 for AsD_3 is found to be 1523.1 cm.$^{-1}$

If the values Be (and therefore $I_{zz}^{(e)}$ are known for both the molecules AsH_3 and AsD_3 it is possible to determine the two quantities r_0(As-H) and the angle between r_0 and the axis of symmetry, β. From these we may then determine the values $I_{aa}^{(e)}$ and the reciprocals of inertia $Ce = h/8\pi^2 cI_{aa}^{(e)}$. For Be we shall here use the values B'' for the molecules in the normal state. We obtain for r_0 the value $r_0 = 1.5126$ A and for the value $\beta = 54° 39'$. For C'' the two values $C'' = 3.663$ cm.$^{-1}$ and $C'' = 1.831$ cm.$^{-1}$ are obtained, respectively, for the molecules AsH_3 and AsD_3.

With these values for B'' and C'' an attempt has been made to analyze the band ω_2 for AsH_3. The details of this will be deferred for a later communication, but satisfactory agreement is obtained with experiment when the values $\nu_2 = 2123$ cm.$^{-1}$, $B' = 3.69$ cm.$^{-1}$, $(1 - \zeta_2)\, C - B = 0.16$ cm.$^{-1}$ and $(B' - B'' - C' + B'') = 0.02$ cm.$^{-1}$ are adopted. These calculations lead to an approximate value of $\zeta_2 = -0.0602$ for AsH_3.

Figure 3 shows the 10 μ region of absorption for AsH_3. This region we believe is also made up of two bands, namely, ω_3 and ω_4 using as before the notation of Dennison. ω_3 is a vibration which induces an electric moment parallel to the axis of symmetry. We identify the band center 905 cm.$^{-1}$ with ν_3 because of the two Q branches and the band center 1002.6 cm.$^{-1}$ as ν_4. No attempt has been made to analyze either the bands ν_3 or ν_4, but it is found that in the R branch of ν_4 and the P branch of ν_3 the spacings between the R and P lines are, respectively, about 10 cm.$^{-1}$ as compared to a spacing of about 7.0 cm.$^{-1}$ in the bands ν_1 and ν_2. An explanation of this anomaly of rotational spacing is found in a strong Coriolis coupling between the frequencies ω_3 and ω_4. The theory of such an interaction has been developed by one of us[2] and applied in a general way to the case of AsH_3[3] in an earlier note. An attempt to analyze this region in detail will be made at a later time.

The region corresponding to figure 3 for AsD_3 is not available. Measurements were made on this region, but it appears that the sample used for this set of measurements was contaminated with some of the hybrid molecules $AsHD_2$ and AsH_2D. This region we hope to repeat at a later date. For ν_3 and ν_4 for the AsD_3 molecule we take the values given by Lee and Wu.[1] Their values are $\nu_3 = 660$ cm.$^{-1}$ and $\nu_4 = 714$ cm.$^{-1}$.

If the normal frequencies ω_2 and ω_4 are known, and not just the band centers, it is possible to calculate ζ_2 for AsD_3 and compare the computed value with the experimental value. A method for closely simulating the normal frequencies for molecules like AsH_3 and AsD_3 has been given by

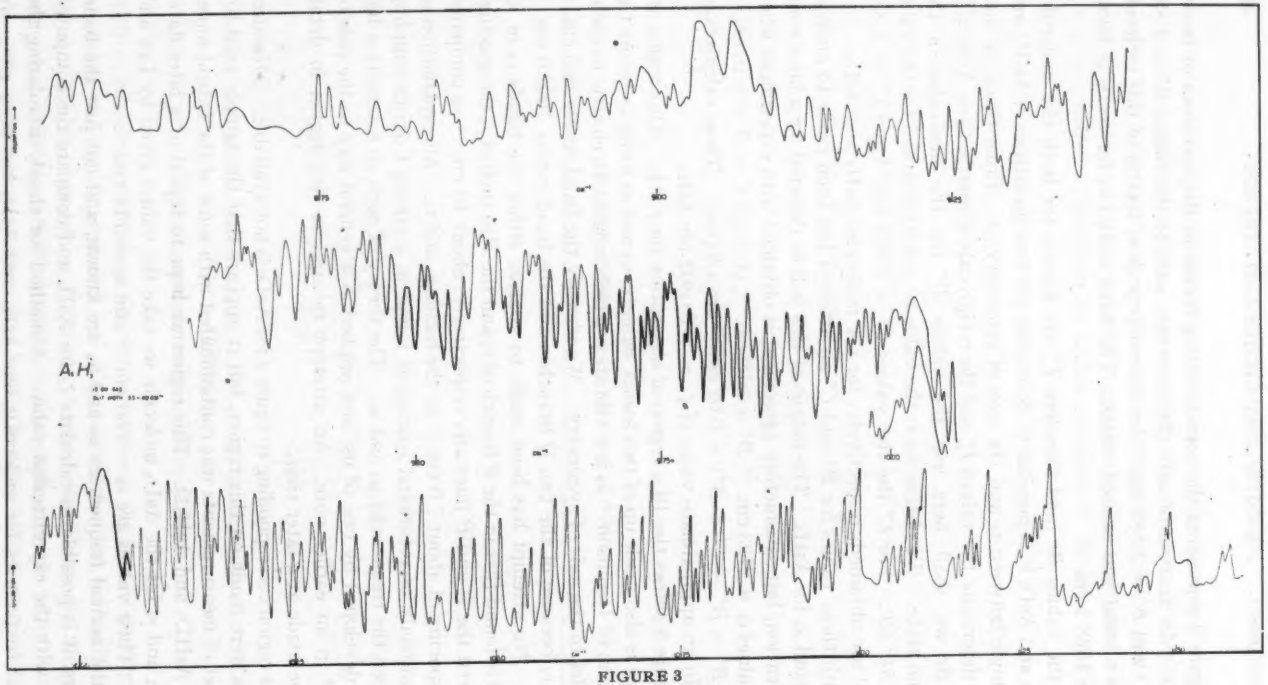

FIGURE 3

ω_2 and ω_4 in the infra-red spectrum of AsH_3.

Dennison[4] and this method is applied here. For ζ_2 for the AsD_3 molecule $\zeta_2 = -0.0383$ is obtained. For the value $(1 - \zeta_2)C - B$ a value 0.0053 cm.$^{-1}$ is predicted. This value seems consistent with the measurements on ω_2 for that molecule which shows that the lines are about equally sharp on both the high- and the low-frequency side of the band. For this to be true $(1 - \zeta_2)\ C - B$ itself must be very small as well as the value $B' - C' - B'' + C''$.

It will be seen that the angle β obtained for the AsH_3 molecule in this work is considerably smaller than the value derived by Sutherland, Lee and Wu.[5] For this reason it is worth while to make an independent verification of this value. We may assume that the force fields of the molecule are closely approximated here by valence forces. On the basis of valence force fields Lechner[6] has derived certain equations which relate the normal frequencies to the valence force constants, the atomic masses and the angle θ. We state one of these.

$$4\lambda_2\lambda_4 = \frac{f}{m}\frac{6d}{m}\frac{2}{1 + 3\cos^2}\left[\rho + (2 - \rho)\cos^2,\right] \qquad (1)$$

where $\rho = \dfrac{3m + M}{M}$, M and m being the masses of the As and H atoms

TABLE 1

BAND CENTERS AND NORMAL FREQUENCIES FOR AsH_3 AND AsD_3

MOLECULE	ν_1	ν_2	ν_3	ν_4	ω_2	ω_4
			904.4			
AsH_3	2116.1	2123.0	906.9	1003	2225.8	1012.1
AsD_3	1523.1	1529.3	...	714	1528.4	718.6

TABLE 2

ROTATIONAL CONSTANTS AND MOLECULAR DIMENSIONS OF AsH_3 AND AsD_3

MOLECULE	$I_{zz} \times 10^{40}$	Bo	$I_{xx} \times 10^{40}$	Co	ro	ho	
AsH_3	7.517	3.723	7.641	3.663	1.513 A	0.93 A	54° 39′
							54° 15′
AsD_3	14.76	1.896	15.282	1.831	1.513 A	0.93 A	54° 39′
							54° 15

and where $\lambda_i = 4\pi^2 c_2^2 \omega_i^2$, ω_i being one of the normal frequencies. The normal frequencies ω_2 and ω_4 for the two molecules AsH_3 and AsD_3 have been found to take the values $\omega_2 = 2225.8$ cm.$^{-1}$, $\omega_4 = 1012.1$ cm.$^{-1}$, $\omega_{2'} = 1582.4$ cm.$^{-1}$ and $\omega_{4'} = 718.6$ cm.$^{-1}$. Since f, d and β will be the same in the two molecules one may quickly arrive at the relation

$$\frac{\lambda_2\lambda_4}{4\lambda_{2'}\lambda_{4'}} = \frac{\rho + (2 - \rho)\cos^2\beta}{\rho' + (2 - \rho')\cos^2\beta}. \qquad (2)$$

Using the above values for ω_i one quickly arrives at the value $\beta = 54° 15'$ which is in satisfactory agreement with the value arrived at earlier. This

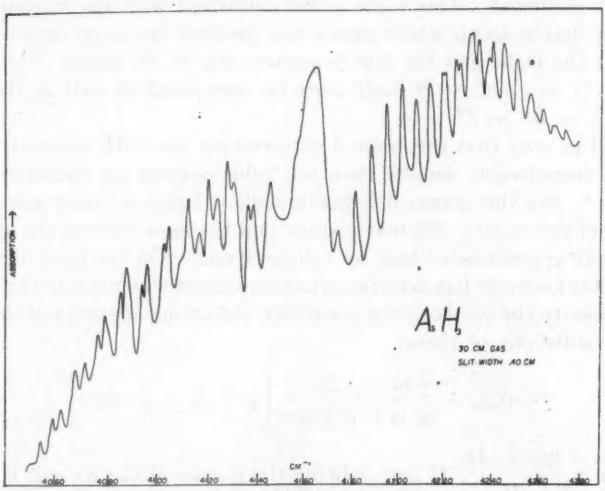

FIGURE 4

Absorption band in the spectrum of AsH_3 near 4160 cm.$^{-1}$.

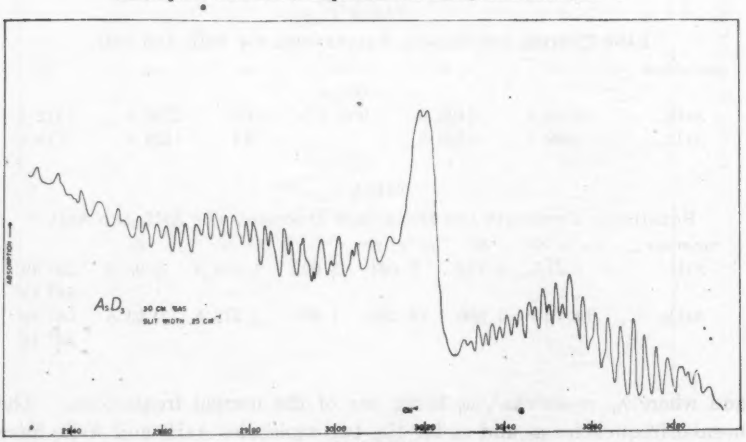

FIGURE 5

Absorption band in the spectrum of AsD_3 near 3020 cm.$^{-1}$.

calculation serves to illustrate how important it is to use the normal frequencies rather than the band centers in determining the constants of a molecule from vibration spectra.

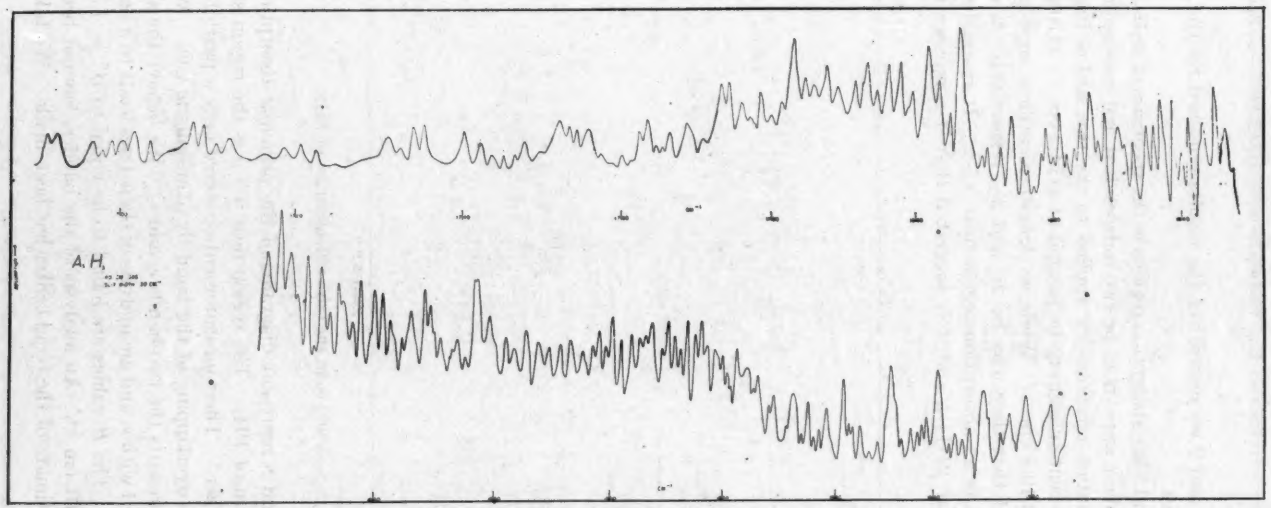

FIGURE 6

Absorption band in the spectrum of AsH_3 near 1800 cm.$^{-1}$.

In tables 1 and 2 we summarize the results obtained for the AsH_3 and AsD_3 molecules.

Figures 4 and 5 are absorption regions in the spectrum of AsH_3 and AsD_3. In each case there appears to be two independent and overlapping bands. If the combination relations are applied to the two sets of lines in each case the band centers seem to be located at 4174.2 cm.$^{-1}$, 4170 cm.$^{-1}$ and 3022.2 cm., 3019.8 cm.$^{-1}$. While no definite identification is given here it is suggested that they may be $2\nu_2$ and $2\nu_1$, respectively, in each case. Figure 6 depicts an absorption region near 5.5 μ. It resembles strongly the region near 10–11 μ and it is suggested that it comprises the bands $2\nu_3$ and $2\nu_4$.

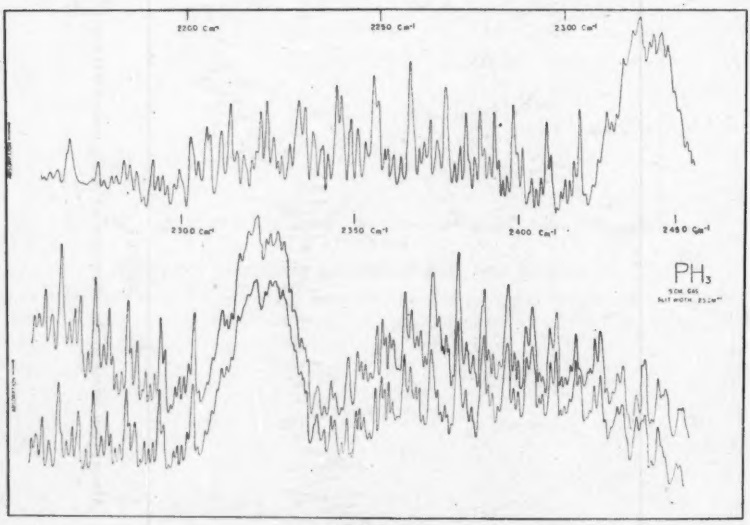

FIGURE 7

ω_1 and ω_2 in the infra-red spectrum of PH_3.

Figures 7 and 8 represent diagrams of the principal absorption regions in the spectrum of PH_3. The region near 4.3 μ is the region studied by Fung and Barker.[7] Their measurements covered only a part of the band because of the overlapping of the band by atmospheric CO_2. We believe this region represents the two bands ν_1 and ν_2. We believe the sharp lines to be associated with ν_1 and an analysis of these lines leads to a band center 2322.9 cm.$^{-1}$. The B values are found to be equal to $B'' = 4.358$ cm.$^{-1}$ and $B' = 4.301$ cm.$^{-1}$. An analysis of the band ν_2 has not been carried out, but an estimate of the band center has been made. We take it to be 2328 cm.$^{-1}$.

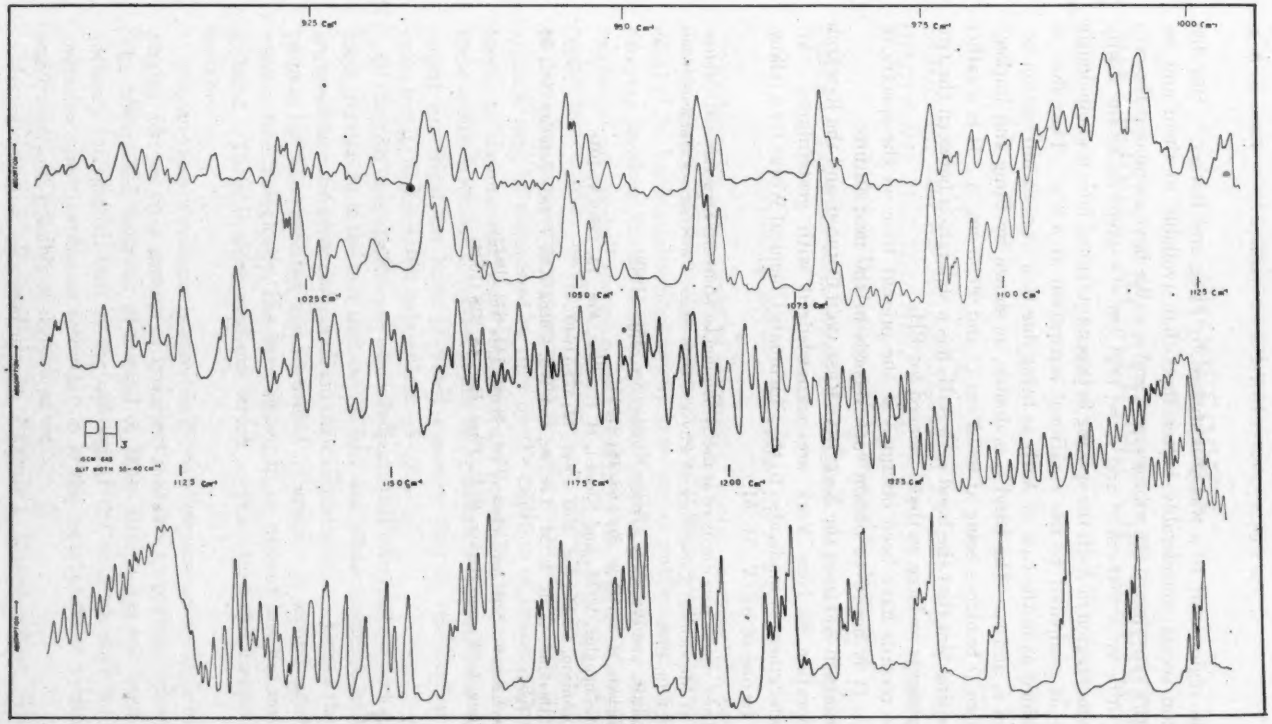

FIGURE 8

ω_3 and ω_4 in the infra-red spectrum of PH_3.

The region near 10 μ was also studied by Fung and Barker.[7] Our dispersion seems considerably better than that available to them and we identify it as due to the vibrations ν_3 and ν_4. We have estimated the centers to be $\nu_3 = 990$ cm.$^{-1}$ and $\nu_4 = .1121$ cm.$^{-1}$. Here as in the corresponding region in AsH_3 the spacing between rotational lines is anomalously large as compared to the rotational separation at 4.3 μ. The effect is explained as in the case of AsH_3 as being due to a Coriolis interaction between ν_3 and ν_4. The band ν_3 is double, as shown by Fung and Barker,[7] the two Q branches being at 989.9 cm.$^{-1}$ and 992.5 cm.$^{-1}$. It is a rather surprising fact that the band ν_3 in AsH_3 has a separation between the two components as large as that observed for PH_3.

No records have been obtained at the present time on the spectra of PD_3. It is hoped to obtain such records in the near future.

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[1] Lee, E., and Wu, C. K., *Trans. Faraday Soc.*, **35**, 1 (1939).

[2] Nielsen, H. H., *Phys. Rev.*, **68**, 181 (1945).

[3] McConaghie, V. M., and Nielsen, H. H., *Phys. Rev.*, **73**, 1250(A) 1948.

[4] Dennison, D. M., *Rev. Mod. Phys.*, **12**, 175 (1940).

[5] Sutherland, G. B. B. M., Lee, E., and Wu, Cheng-Kai, *Trans. Faraday Soc.*, **35**, 1374 (1939).

[6] Lechner, F., *Setz. ber. Akad. Wiss., Wien*, **141**, 633 (1932).

[7] Fung, L. W., and Barker, E. F., *Phys. Rev*, **45**, 238 (1934)